

STUDIES IN CAMBIAL ACTIVITY

1. CAMBIAL ACTIVITY, ROOT HABIT AND SUCKER SHOOT DEVELOPMENT IN TWO SPECIES OF POPLAR.
11. CAMBIAL ACTIVITY IN POPLAR WITH PARTICULAR REFERENCE TO POLARITY PHENOMENA.
111. ACTIVITY OF THE VASCULAR CAMBIUM IN RELATION TO WOUNDING IN THE BALSAM POPLAR.

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BY

ALEXANDER BINNIE BROWN, B. SC. (EDIN.), M. SC. (M^CGILL).

Lecturer in Botany at the University of Alberta, Edmonton, Canada.

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A. B. BROWN

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CAMBIAL ACTIVITY IN POPLAR
WITH PARTICULAR REFERENCE TO POLARITY PHENOMENA

BY

A. B. BROWN,
LECTURER, DEPARTMENT OF BOTANY,
UNIVERSITY OF ALBERTA.

ABSTRACT

7- Ringing experiments with the aspen poplar, Populus tremuloides Michx., lead to the conclusion that cambial activity is definitely not rigidly/or unconditionally polar in its development in the root. A much greater development of cambial activity in the morphologically upward direction was obtained in these experiments than has hitherto been observed. It is suggested that the concept of polarity, applied to cambial activity as a process, must be defined in terms of a tendency to develop in the morphologically downward direction, rather than in the morphologically upward direction, in roots and stems. Polarity in relation to cambial activity in general is discussed briefly.

INTRODUCTION

In a previous communication, the writer (1) showed that cambial activity emanating from sucker shoots of poplar flows apparently almost entirely in the distal direction on entering the parent root, resulting in marked thickening of the root on the distal side, except in cases where sucker shoots occur very close to one another, when this distal thickening may not be obvious. This type of behaviour is expressed invariably however in the grain of the wood, which runs longitudinally down the sucker shoot and continues likewise along the root in the distal or acropetal direction.

In the crotch, i.e. the region common to both shoot and root, the grain runs longitudinally at all points on the distal side, but on the proximal side it divides, turns sharply through an angle of 90° , and then swings round to run longitudinally and distally. As a result, in longitudinal radial sections of the root and sucker shoot at the crotch, the xylem elements are cut longitudinally on the distal side, whereas on the proximal side, in the region common to both root and shoot, the xylem elements are cut more or less transversely. It was also shown that the tissue orientations just described are anticipated at a very early stage; viz., around the base of the sucker bud from which the sucker shoot ultimately develops. Vascular connection between the sucker bud and the root xylem is attained by the development of a peg of tracheids. As a result of the interaction between the normal acropetal flow of cambial activity in the root and this vascular peg, the tissues subsequently formed turn sharply on the proximal side and orientate themselves around the peg, as seen in longitudinal tangential sections, in a form essentially similar to that of a flow-pattern. In longitudinal radial section, the root xylem is cut transversely just proximal to the vascular peg and longitudinally on the distal side.

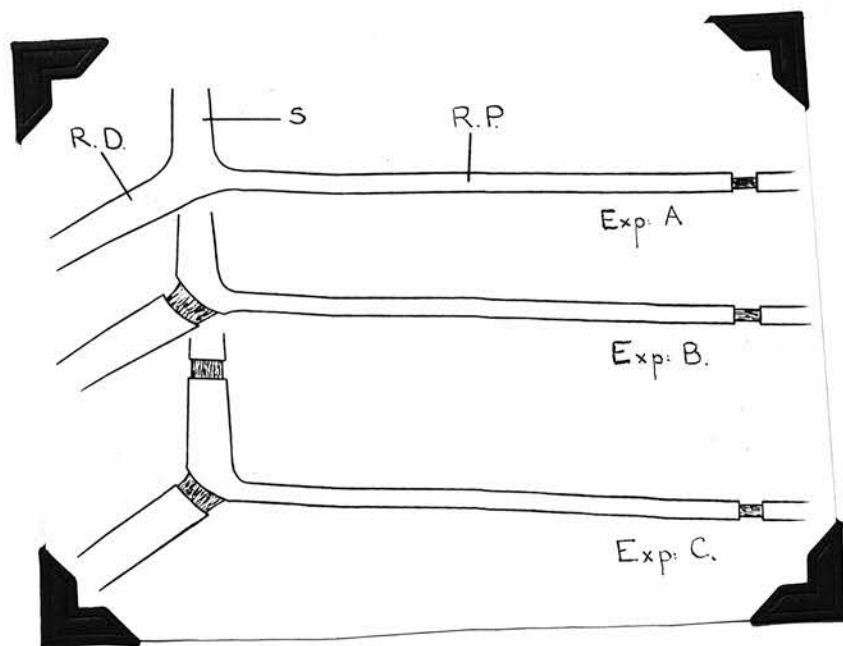
Now in the early part of the summer, soon after the basipetal flow of cambial activity from the shoot has reached the root, a well defined gradient of cambial activity, estimated in terms of xylem formation, can be detected running from the base of the shoot along the root in the distal direction. At the same time however, a feeb-

le gradient in the opposite direction can be observed, running from the base of the shoot along the root in the proximal direction. Certain investigators, who will be referred to later, postulate that cambial activity is polar, in so far as it can only travel in the morphologically downward direction in the stem, and in the acropetal direction in the root. However, in view of the gradient of xylem formation in the proximal direction, feeble though it may be, it would appear that polarity of cambial activity is not complete and unconditional. It was thought worth while therefore, to further investigate the question of polarity of cambial development in this material, and the following experiments were designed expressly to yield some information on this subject. The fact that cambial activity is evidently not completely polar in development suggested to the writer (1) the possibility that the flow of cambial activity from the shoot may actually be guided along the root in the distal direction, as a result of the reorientation of the tissues around the vascular peg at the base of the sucker bud, from which the shoot arises. In other words, the phenomenon could conceivably be explained, in a very simple manner, without postulating polarity. On the other hand, it should be remembered that there are a number of phenomena indicative of polarity of cambial activity that could not be so explained.

EXPERIMENTS AND RESULTS

Part 1: Extent of development of cambial activity in the proximal direction in roots under experimental conditions.

The material used was aspen poplar, Populus tremuloides Michx., and three different experiments, A, B and C, (text - fig. 1) were performed. In experiment A the root was completely ringed some distance proximal to the sucker shoot, in such a way as to remove all the tissues external to the xylem. In experiment B, two complete rings were made, one proximal to the sucker shoot just as in experiment A, and one immediately distal to the sucker shoot. In experiment C three rings were made, one some distance proximal to the sucker, one immediately distal, and the third a short distance up the shoot. All growing points, if any, below the stem ring were removed. It was found that unless the ring was made quite close to the base of the stem, death of the parts above the ring ensued comparatively soon, whereas when the stem ring was near the base, the parts above could remain alive until the end of the growing season. In these experiments, the distance between the crotch and the proximal ring varied from 6 to 40 inches and was determined simply by the habit of the material. The sucker shoots varied in age from 5 to 14 years, and the operations were carried out within the last two weeks in May and the first week in June in the years 1934 and 1935. At that time new xylem formation had not commenced in the roots. In all, about 100 trees were treated. The roots were uncovered as care-



Text - figure 1.

Text - fig. 1. Illustrating the relative positions of the complete rings in experiments A, B and C. S = the sucker shoot, R.D. = the root distal to the shoot and R.P. = the root proximal to the shoot.

fully as possible, and the operations performed on the trees as they occurred in situ. The exposed xylem in the rings was thoroughly scraped with a scalpel, and the wound then rubbed with vaseline and finally covered with grafting wax. No healing over of the wounds ever took place. Usually the exposed roots were covered with loose turf after the operation. There was a marked tendency for sucker buds to arise just distal to the rings in the root, particularly the ring proximal to the sucker shoot. This tendency was not so evident just distal to the ring immediately distal to the shoot. These buds were usually removed whenever they were detected.

Subsequent growth of the treated trees was quite normal in the case of experiment A. In B and C treated plants, the leaves tended to be smaller than usual, and took on a bronze colouration at an early date. However, it was not at all uncommon to find trees receiving the B treatment perfectly normal in appearance with regard to leaf size and colour. No difference in time was observed with respect to leaf fall at the end of the season. A few A and B treated specimens were left to overwinter and in the following spring they all leafed out at the same time as untreated trees. In most of the A treated trees which had overwintered, the root proximal to the sucker was found to have died, either during the winter or early in the second season. Otherwise the A treated trees were perfectly normal during the second season. In B material the leaves were smaller and bronzed early in all the specimens that had overwintered, and there appeared to be a definite reduction in extension growth during the second season. The

specimens were still alive on August 9, 1935, when all of them were collected.

With regard to cambial activity, estimated in terms of xylem formation, the results can be summed up as follows: A falling gradient of xylem formation in the root on the side proximal to the sucker shoot was obtained in all three experiments. It was least marked in C material, most marked in B, while A was intermediate. The following estimations of the width of the annual ring at different distances from the sucker shoot in four experiments, A 14(24"), A 16(16"), B 3(14") and B 13(37"), will indicate the type of xylem gradient obtained in experiments A and B. The figure in brackets after the number of the experiment indicates the length in inches between the shoot-root crotch and the ring proximal to the crotch. All widths are compared with the width of the annual growth ring for the current year in the shoot, at a distance of six inches from its base, and this is arbitrarily rated at one hundred in all cases. In no experiment were there any growing points on the shoot lower than six inches from its base. The actual widths of the annual ring in the shoot, six inches from the base, in A 16, A 14, B 3 and B 13, were, in arbitrary units, 140, 40, 32 and 37 respectively. It will be observed that there was very little difference between the widths in A 14, B 3 and B 13, so that the figures for these three experiments can be compared with one another in terms of actual width without any great error.

Experiments A 16 and A 14

	<u>Treated</u>	<u>Collected</u>
A 16	3/6/35	9/8/35
A 14	3/6/35	4/9/35

<u>Width of Annual Ring</u>	<u>A 16</u>	<u>A 14</u>
Stem: 6" from base	100	100
Root: 1" distal to shoot	150	136
" 6" " " "	150	69
Root: 1" proximal to shoot	63	36
" 3" " " "	49	29
" 6" " " "	38	26
" 9" " " "	30	22
" 12" " " "	25	19
" 15" " " "	15	16
" 18" " " "		13
" 21" " " "		10

Experiments B 3 and B 13

	<u>Treated</u>	<u>Collected</u>
B 3	29/5/35	9/8/35
B 13	3/6/35	9/8/35

<u>Width of Annual Ring</u>					<u>B 3</u>	<u>B 13</u>
Stem:	6" from base				100	100
Root:	1" proximal to shoot				150	324
"	3"	"	"	"	103	216
"	6"	"	"	"	75	108
"	9"	"	"	"	58	117
"	12"	"	"	"	50	102
"	15"	"	"	"		95
"	18"	"	"	"		95
"	21"	"	"	"		102
"	24"	"	"	"		102
"	27"	"	"	"		87
"	30"	"	"	"		70
"	33"	"	"	"		50
"	35"	"	"	"		27

It is clear from the above results that a very appreciable gradient of cambial activity was laid down in the root proximal to the sucker shoot, in a system otherwise untreated except for a complete ring some distance proximal to the shoot (experiments A 16 and A 14). There was no question of any stimulus passing across the ring, for precisely the same result obtained if the root was cut through completely, instead of ringed. Apparently, therefore, cambial activity is not rigidly polar in its development in the root. In experiment B the gradient of xylem, laid down in the root on the proximal side, was not less and usually greater than that found on the distal side in untreated, or A treated material. On the whole, it was found that the gradient on the proximal side in B material fell off rather more rapidly than it did on the distal side in A material. There was no indication whatsoever of any reorientation of the tissues in the shoot-root crotch, despite the fact that the distal ring was shaped (text - fig. 1), with the aim in view of facilitating any changes that might tend to take place.

Now it is well known that a basifugal development of cambial activity takes place locally from the upper edge of a complete ring in a shoot, quite independent of developing buds or elongation growth. Precisely the same sort of behaviour occurs in roots and experiment C yielded information on this point. It will suffice to state that the gradient of xylem formation obtained in the proximal direction was exceedingly feeble. Several rows of xylem elements might be laid down just proximal to the shoot, but at a very short distance proxi-

mal, about 4 inches, the gradient practically faded out. The amount of xylem laid down proximal to the shoot in B material was, without a doubt, much greater than could be accounted for, if it were compounded simply of the amount of xylem formed as a result of A treatment plus the development resulting from C treatment.

A word now about the anatomical features of the new xylem laid down proximally in the root in these experiments. Almost invariably in experiments A and B, the xylem was denser than that of previous years in the root. This was due principally to the fact that the vessels were smaller. Indeed, the new wood rather resembled typical stem wood. Occasionally one found a little parenchyma, formed at the beginning of cambial activity, but the new wood was not at all characterised by the presence of parenchyma as a constant feature, except towards the end of the gradient where it approached the ring proximal to the shoot. Here the wood invariably did include an abundance of parenchyma. Otherwise the wood laid down proximally was quite normal, consisting of vessels, tracheids, fibres and medullary rays. In experiment C the new xylem did appear to be rather atypical. The conducting elements were again smaller in diameter, they often occurred in groups and consisted chiefly of tracheids rather than vessels, which although present were rather scarce. Moreover, the fibres were not thickened to nearly the same extent as in experiments A and B. The wood laid down immediately distal to the shoot in normal untreated roots is usually rather denser and more like stem wood than that found at points further removed from the shoot, but it was observed in experiment A that the current year's

wood distal to the shoot was not infrequently denser than that laid down in previous years. Moreover, one occasionally did find cases of A and B experiments, where the wood formed in the root proximal to the shoot was not markedly denser than the previously formed wood, particularly beyond the immediate vicinity of the shoot. It is quite possible that the formation of denser wood in the roots in these experiments was determined to a certain extent by some such factor as water supply. A large percentage of the feeder roots were inevitably destroyed during excavation prior to operation, and normal translocation of water would very probably be upset as a result of ringing, particularly in experiments B and C. The wood laid down in the shoot in experiments A and B was quite typical and did not appear to differ at all from that of previous years. A point of considerable interest is that the width of the post-operative growth ring in the shoot was sometimes greater and sometimes less than that of the previous year in both A and B experiments. In B material however, there was a marked decrease in width of the growth ring in the second season after operation, i.e. in trees that had overwintered.

Figures 1, 2 and 3 (Plate 1) illustrate the type of development obtained in three experiments A 1, B 23 and C 1. They show clearly that the wood formed in the root proximal to the shoot after the operative procedure is markedly denser than that of previous years. Moreover they give some idea of the extent of development that can be obtained in experiments A and B at a considerable distance, 7 inches, proximal to the shoot-root crotch. In C 1 the section illustrated was

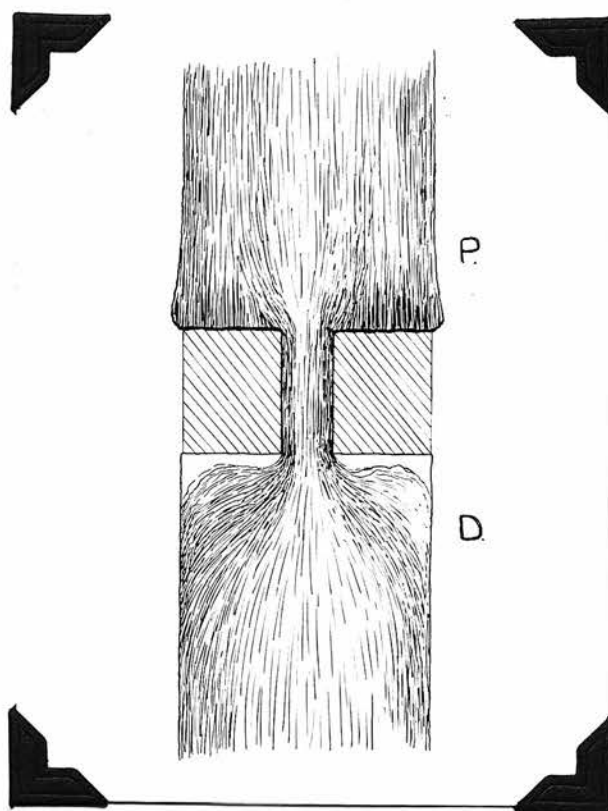
cut at slightly less than 1 inch from the crotch; at a distance of 7 inches from the crotch no new xylem had been formed. It was found, in all the B experiments, that for at least a considerable distance proximal to the shoot, the current year's growth ring was wider than any other growth ring laid down in that region previous to the operation. This applied only to the first post-operative growth ring. As has already been pointed out, development in the second season after operation was rather feeble. In experiment A 1 (Plate 1, Fig. 1) the current year's growth ring is wider than any of the others shown, but not so wide as some of the rings nearer the centre of the section and not included in the photograph. The only generalisation that can be made with regard to the A experiments is that the width of the growth ring formed after the operation was not always less than that of previously formed rings. As far as the writer is aware cambial activity in the proximal or morphologically upward direction has not hitherto been observed to the extent obtained in either experiment A or B, with roots of poplar.

Part 2: Tissue orientations in relation to a wound when cambial development is proximal in the root.

In a woody stem, if instead of making a complete ring a longitudinal phloem bridge is left, it is found that cambial activity accumulates or "piles up" immediately above or distal to the wound, flows through the bridge and then spreads out downwards and obliquely round the stem below the wound (text - fig. 2). Transverse sections just above the wound show the xylem to be cut transversely at all

points, except where the elements turn in slightly towards the bridge where they may be cut very slightly obliquely, whereas just below the wound the xylem is cut transversely only in the same longitudinal line as the bridge, and definitely obliquely at all other points. It is not proposed to discuss this type of behaviour in any detail in this paper; what is of particular interest is that it may be considered to be a typically polar phenomenon. Precisely the same type of behaviour obtains in similarly wounded roots. The accumulation or "piling up" of xylem formation occurs on the proximal side of the wound, and the spreading out on the distal side. Wounds of this type were accordingly made in experiments A and B, in that region of the root between the sucker shoot and the proximal complete ring, at a time when the cambium was still active and after a considerable gradient of xylem formation had been laid down in the proximal direction. In every case the orientation of the tissues subsequently formed in relation to the wound was absolutely normal.

There was no reversal either in experiments A or B. The following analysis of experiment A 5 illustrates the type of result obtained. There were two dates of treatment, the first when the complete ring proximal to the shoot was made and the second when the wound with the longitudinal phloem bridge was made. The second wound was located at about 7 inches from the crotch and the phloem bridge was somewhat less than 1 inch long. Note that the point 7 inches proximal to the shoot was really distal to the wound and the point 8 inches proximal to the shoot really proximal to the wound, in terms of location on the root.



Text - figure 2.

Text - fig. 2. Tissue orientations in the wood of a shoot or root in relation to a ring with a longitudinal phloem bridge remaining. P = the root proximal to the wound or the shoot above the wound, D = the root distal to the wound or the shoot below the wound.

Experiment A 5

1st treatment 12/5/34 Collected 23/7/34

2nd " 3/7/34

Width of annual ring

Stem:	3" from base	100	
Root:	2" distal to shoot	112	
"	6" " " "	93	
Root:	1" proximal to shoot	39	
"	2" " " "	35	
"	3" " " "	32	
"	4" " " "	31	0 to 7
"	5" " " "	27	7 to 14
"	6" " " "	24	14 to 17
"	7" " " "	23	1 to 27 (a)
"	7.5" " " "	21	37 (b)
"	8" " " "	21	20 to 27 (c)
"	9" " " "	19	13
"	10" " " "	27	(= 18 + 9)
"	11" " " "	19	(= 16 + 3)
"	12" " " "	15	

(a) immediately distal to bridge

(b) within bridge

(c) immediately proximal to bridge

It is clear from the above analysis that wounding has brought about a marked increase in cambial activity in the vicinity of the wound. Moreover, the first xylem formed after wounding consisted largely of vessels, so that what was really a false growth ring developed, enabling one to measure the extent of development prior to and subsequent to wounding. A few inches proximal to the wound, the demarkation between the wood formed prior to wounding and that formed after was not clear (this seems to be a constant feature), and the figures in brackets at 10 and 11 inches proximal to the shoot are rough extrapolations. As has already been stated, the tissue orientations around the wound were quite normal, no reversal having taken place. This is clearly indicated in the figures. Just proximal to the phloem bridge the new wood laid down after wounding was but slightly excentric (20 - 27) and the elements were all cut transversely, whereas the new wood just distal to the bridge was very markedly excentric (1 - 27) and was cut transversely only in the same longitudinal line as the bridge, and obliquely at all other points as it spread downwards and obliquely round the root. The width of the new wood laid down in the bridge itself subsequent to wounding was always greater than that immediately distal or proximal to the wound. This was a constant feature. Precisely the same results were obtained in the B experiments. Figure 4 (Plate 1) depicts a transverse section of the root within the bridge in experiment B 2, and shows the extent of development prior to and subsequent to wounding, at the time of collection. The results were

definite and absolutely invariable, even when the wound was little more than 1 inch from the crotch. Moreover the same result was obtained in four B experiments, with trees that had overwintered and in their second season of growth after the first operative treatment. Here again, there was no reversal of the orientation of the tissues around the wound with the longitudinal phloem bridge. Whether or not similar results would be obtained in the third season of growth is not known. B material did rather poorly in its second season. There was less extension growth, fewer leaves produced, and a marked decrease in the amount of cambial activity. No experiments were performed with A material in the second season. Of the few that were left to overwinter, in most cases the root proximal to the sucker had died or was otherwise unsuitable for further treatment. The wood formed subsequent to wounding in these experiments was apparently quite normal. In experiment B 2 (Plate 1, fig. 4), the wood formed after wounding was somewhat denser than that laid down before wounding, but this was not always the case.

The complication arising from the above results is probably obvious. It is simply this, that there was no reversal of the tissue orientations around the wound with the longitudinal phloem bridge, under conditions where such a reversal might reasonably have been expected. The development of cambial activity is normally basipetal in the shoot and acropetal in the root, and the orientation of the tissues with relation to a wound with a longi-

tudinal phloem bridge, in an otherwise untrested shoot or root (text - fig. 2), could very naturally be interpreted as simply a manifestation of the normal mode of development. From this point of view therefore, the fact that there was no reversal in experiments A and B is rather surprising. There is however at least one point that may be of considerable significance in this connection. If a wound of the type under consideration is made in a dormant shoot or root, cambial activity is initiated locally around the wound, quite independent of cambial activity emanating from developing buds, which may indeed be entirely removed. Moreover, the new tissues orientate themselves in the vicinity of the wound precisely in the manner described above. Apparently to get cambial activity as a result of wounding, the phloem must be cut in such a way as to cause discontinuity of the elements. Any transverse or oblique incision would bring this about, whereas a longitudinal incision possibly would not, if the elements were running strictly longitudinally. In addition cambial activity only occurs if the cut has phloem tissue immediately distal to it in the stem, or proximal to it in the case of the root. This is clearly expressed in the fact that, if a complete ring is made in a stem, cambial activity leads to the production of a basifugal gradient of xylem from the upper margin of the ring, whereas no cambial activity is evident at the lower margin. It is therefore not inconceivable, that the orientation of the tissues superimposed upon the proximal gradient in experiments A and B was governed by the phloem tissue formed before the

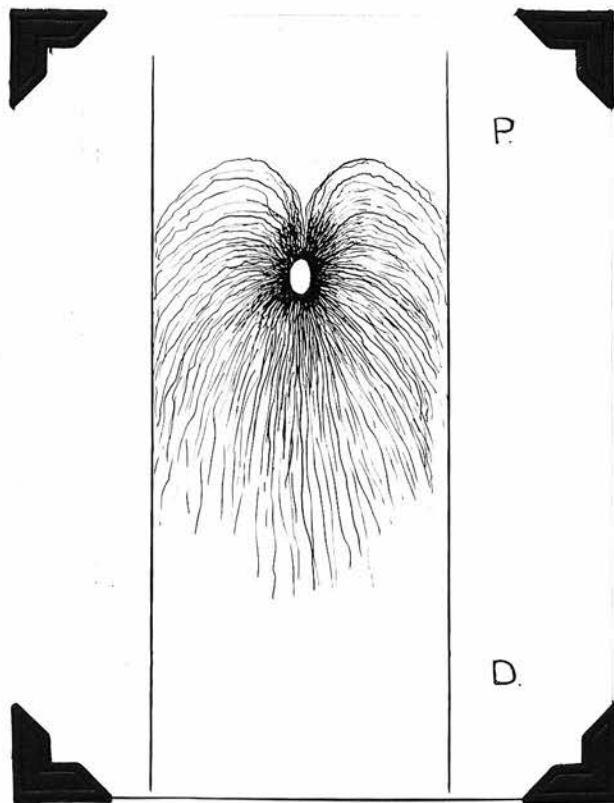
first operative procedure, despite the fact that a certain amount of new phloem had also been laid down, presumably in the same manner as the xylem, in a proximal gradient. But on the other hand there was no evidence that the amount of cambial activity, superimposed upon the proximal gradient in the vicinity of the wound with the longitudinal phloem bridge, was determined by the phloem formed previous to the first operative procedure, since it was always greater relative to the amount of cambial activity obtained in the C experiments.

Part 3: Relationship between cambial activity and growth of sucker buds, in regions of the root where the normal distal development of cambial activity is not taking place.

During the course of the above experiments, the writer was able to make observations of marked significance with relation to the behaviour of sucker buds, which, if they did not arise definitely after, at least had not made vascular connection with the root xylem prior to the operative procedure. These observations apply to such buds arising distal to the distal complete ring in experiment B, or between two complete rings in regions of the root having no phloem connection with sucker shoots, as in experiment C, and making vascular connection with the parent root in regions where the cambium was not dividing and the normal acropetal flow of cambial activity not present. Text - figure 3 illustrates the manner in which the new xylem resulting from bud activity is laid down upon the surface of wood of the previous year, as observed in pieces of root which

had been peeled and allowed to dry out. Generally speaking, the new wood runs acropetally downwards and obliquely round the surface of the old wood. But just proximal to the bud, the new wood actually runs almost directly proximal for a short distance before it turns. The writer has already pointed out that sucker buds tended to arise just distal to a complete ring, but wishes to emphasise that the behaviour illustrated was commonly observed at points considerably removed from any complete ring. Behaviour of similar buds close to a complete ring was however the same. Precisely the same type of behaviour was observed close to the complete ring proximal to the shoot in experiments A and B, again with buds that had not attained vascular connection with the root prior to operation. Here, surprisingly enough, the wood resulting from bud activity spread distally or acropetally upon the surface of wood that had been, or was being laid down in a gradient in the opposite or proximal direction. It is true however, that these buds were always observed very close to the complete ring, i.e., right at the end of the proximal gradient where the xylem contained an abundance of parenchyma.

A point of interest arising at this time is related to a former statement by the writer (1), to the effect, that the definite "flow pattern" in the root xylem below the sucker bud, under normal conditions, is a manifestation of the reaction between root cambial activity and the obstruction offered by the vascular peg at the base of the sucker bud. In view of the above observations, bud activity of itself could not bring about the formation of such a pattern, and



Text - figure 3

Text - fig. 3. Illustrating the way in which cambial activity emanating from developing buds spreads over the surface of the previously formed wood, in regions of the root where the normal acropetal flow of cambial activity is not present. P = the root proximal to the bud, D = the root distal to the bud.

so the writer's earlier statement receives some support.

Part 4: Similar experiments with poplar stems.

The reader will probably be able, without much difficulty, to picture experiments with stems, essentially similar to those already described with roots. Instead of the shoot-root crotch we have now to consider the branch crotch. Corresponding to experiment A, one complete ring was made in one member of the crotch and this limb was completely disbudded below the ring. The other limb was untreated. Corresponding to experiment B, a second complete ring was made immediately below the crotch, and corresponding to experiment C three complete rings were made, one on each of the two limbs and another immediately below the crotch. The material between the rings was completely disbudded. Ringing was carried out during the month of April, before the advent of bud break. The results will be treated as briefly as possible and they all lead to the same conclusion, viz., that cambial activity in any but the basipetal direction was extremely limited in extent. In stem experiment A, the new wood formed in the untreated limb flowed round the base of the ringed limb in a loop, and sometimes there was no basifugal or acropetal development of cambial activity in the ringed limb. Usually, however, there was a slight development acropetally, and occasionally one did find an acropetal development for a few inches above the crotch, in the ringed limb. But at the most, the extent of development was very small. Stem experiment A resembled rather closely the state of affairs obtaining in that method

of pruning where a snag is left above the bud. Wray (12) has shown that in such a case, cambial activity emanating from the bud flows downwards and obliquely around the stem, and there is no development in the acropetal direction in the snag. The snag, of course, dries out rapidly. Knight (5), on the other hand, observed a slight upward development of cambial activity in disbudded apple shoots, from the first growing branch below, and Sledge (7) reports that Swarbrick observed a very slow upward spread of new wood formation in a ringed apple shoot, from the first bud below up to the lower edge of the ring.

In stem experiment B, cambial activity from the limb bearing developing growing points again tended to flow round the base of the ringed limb in a loop, but further progress was of course prevented by the complete ring below the crotch. The ultimate result was usually a marked "piling up" of new wood just above the crotch ring. This was particularly obvious in a thick band of wood running transversely round the base of the ringed limb. Cambial activity in the acropetal direction in the ringed limb was much the same as in stem experiment A. Sometimes there was none at all, usually there was a little, and occasionally cambial activity spread upwards for a few inches. But here again, cambial activity in the acropetal direction was at the most exceedingly feeble. In stem experiment C, a feeble gradient of cambial activity, spreading acropetally upwards in both limbs from the upper edge of the complete ring below the crotch, was the invariable result. Just above the

crotch ring a very appreciable amount of xylem usually formed, but it was "piled up", and the gradient fell off very rapidly. Actually, stem experiment C yielded results very similar to root experiment C. There was however no comparison at all between the extent of development of cambial activity in experiments A and B in stems, relative to that obtained in roots. In the stem experiments, all the tissues above a complete ring invariably died before the end of the growing season.

DISCUSSION

In view of the mode of development of cambial activity emanating from sucker buds, (arising after or at all events having no vascular connection with the parent root prior to the operative procedure, and in regions of the root where the normal acropetal flow of cambial activity is not present) it is to be concluded that the marked development of cambial activity distal to a sucker shoot, relative to that on the proximal side, is not determined by a previous reorientation of the tissues at the base of the sucker bud. The answer, it would appear, is to be looked for in polarity of cambial development, and in this connection a number of points arise for discussion.

Jost (3, 4) was the first investigator to observe that cambial activity in the stem travels only in the basipetal direction. It has also been pointed out at various times by Priestley (6), by Wray (12) by Snow (8, 9, 10) and a few others. Snow (10) also reports that cambial activity develops only in the morphologically downward direction in roots of Vicia Faba. In fact this polar mode of development of cambial activity is now accepted as a general rule, with but few exceptions. However it is with the exceptions that the writer is mainly concerned. The observations of Knight and Swarbrick, of a feeble basifugal development of cambial activity in apple stems under certain conditions, have already been mentioned. These observations are confirmed by the writer's results with poplar stems. Here of course, the upward development of cambial activity is admittedly

very feeble. However, in the case of root experiment A, the development of cambial activity in the proximal direction is by no means feeble, and on the basis of this experiment alone, it would seem that polarity of cambial activity in poplar roots is very definitely not rigid or unconditional, and that any definition of polarity must be couched in terms of a tendency to develop in the distal rather than in the proximal direction. Fundamentally this may also apply to cambial activity in stems. The marked difference in the extent of development basifugally in stems, relative to that proximally in similar experiments with roots, might be conditioned by other factors, for example, water supply. A reasonable explanation of the results obtained in root experiment B is that the cambial stimulus that would normally travel acropetally is diverted proximally, which would also support a definition of polarity in terms of a tendency. The mode of development of a new wood emanating from developing buds under the conditions previously described (text - fig. 3) is of great interest. Actually, cambial activity at first seems to be inherently capable of developing in all directions possible, distally, laterally and proximally, and it looks almost as if the ultimate development distally were being conditioned by some factor not inherent in cambial activity as such.

Reference might also be made to the local basifugal development of cambial activity from the upper edge of a complete ring in stems, and the corresponding development proximal to a complete ring in roots. These are widely recognised exceptions to the general rule in regard to cambial development. But of still greater interest is

the local development of cambial activity in the vicinity of a wound, where instead of making a complete ring a longitudinal phloem bridge is left. In the stem, we get a basifugal development of xylem formation from the upper edge of the wound, similar to that obtained when the ring is complete. Within the bridge development is however basipetal, and just below the bridge it is basipetal and lateral. A corresponding type of development obtains in similarly treated roots. These remarks apply to cambial activity in the vicinity of wounds independent of any normal cambial activity emanating from developing shoots. A more detailed account of cambial activity in relation to wounding will be published later.

Some recent observations by Elliott (2) are also worthy of mention. He has shown that in Acer cambial activity proceeds acropetally into the leaf from the junction of the lamina and petiole, and in Castanea acropetally along the petiole from its base into the lamina. At the same time there is the usual basipetal development of cambial activity down the shoot from the top of the petiole in Acer and from the bottom of the petiole in Castanea.

Now there is a possible danger of the idea of rigid polarity of cambial activity becoming almost a general law in the minds of some investigators, particularly so in view of the fact that Snow and LeFanu (11) have shown that an ether extract of urine promotes cambial activity, and the possibility that the hormone promoting cambial activity may be identical with the auxin causing cell elongation in the oat coleoptile, in which according to a number of investigators translocation of the auxin is strictly polar. The writer suggests in

this connection that the fact that cambial activity does not or apparently cannot proceed in any but the morphologically downward direction in some cases does not necessarily mean that it cannot possibly develop in the opposite direction under any condition.

The fact that there was no reversal of the tissue orientations in relation to the wound with the longitudinal phloem bridge, made subsequent to the first operative procedure in root experiments A and B, is very puzzling. However, a possible explanation has been indicated, and at all events, the writer does not feel that his conclusion, that cambial activity is not rigidly and unconditionally polar, is rendered untenable because of the phenomenon just mentioned.

Recognition of the fact that polarity of cambial activity, which is but one of many polar phenomena in plants, constitutes a difficult problem is of course not new, and no pretence is made that the foregoing experiments indicate a solution of polarity of cambial activity in particular. However definite experimental results have been obtained, on the basis of which the following conclusion is submitted for consideration, viz., that cambial activity is polar in its development, when polarity is defined in terms of a tendency to develop in the morphologically downward direction, rather than in the morphologically upward direction. On the other hand, it may be suggested that cambial activity as a process is not inherently polar in its development, but is determined by some other factor. If however this determining factor should be polarity of the organism as a whole, or polarity of some particular organ as a whole, then the two interpretations

really become identical, and polarity of the organ or organism as a whole would have to be defined in terms similar to those suggested for cambial activity.

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EXPLANATION OF PLATE

- Fig. 1. T. S. root of aspen poplar, seven inches proximal to the shoot in experiment A 1 (13"). Treated 16/5/34, collected 13/8/34. The outermost ring is the post-operative growth ring. X 29.
- Fig. 2. T. S. root of aspen poplar, seven inches proximal to the shoot in experiment B 23 (13"). Treated 15/5/34 collected 13/8/34. The outermost ring is the post-operative growth ring. X 29.
- Fig. 3. T. S. root of aspen poplar, slightly less than one inch proximal to the shoot in experiment C 1 (14"). Treated 16/5/34, collected 13/8/34. The outermost ring which is very narrow is the post-operative growth ring. X 29.
- Fig. 4. T. S. root of aspen poplar in experiment B 2 (30"). Treated 15/5/34, collected 13/8/34. On 3/7/34 a ring with a longitudinal phloem bridge remaining was made in the root about nine inches proximal to the shoot and the section illustrated was cut within the bridge. The xylem formed prior to and subsequent to wounding (3/7/34) is indicated by P and S respectively. X 29.

Note: The figure in brackets after the number of the experiment in the above, indicates the length in inches between the shoot-root crotch and the proximal complete ring.

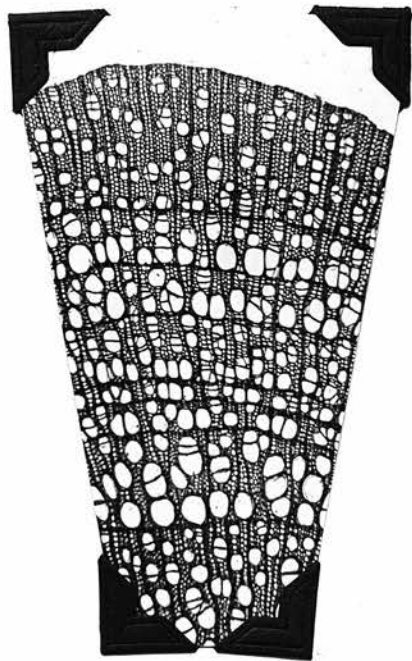
PLATE 1

Fig. 1

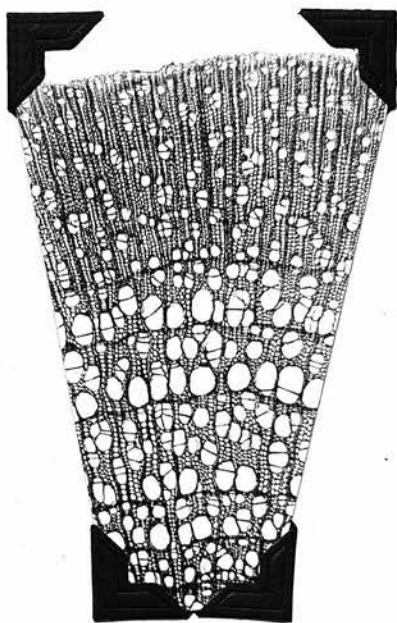


Fig. 2

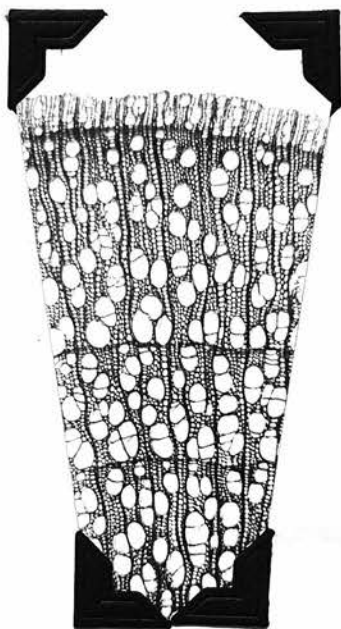


Fig. 3

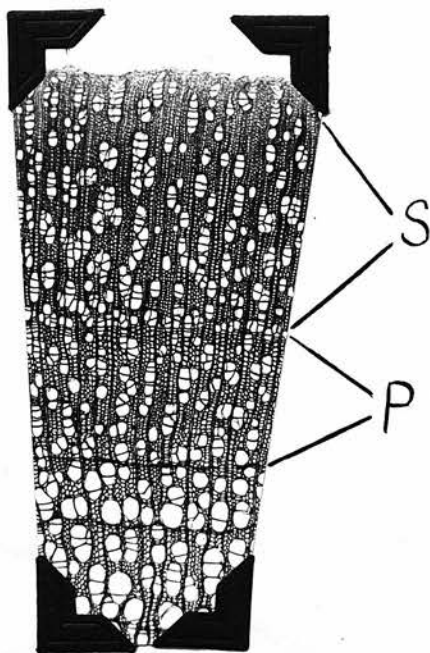


Fig. 4

111. ACTIVITY OF THE VASCULAR CAMBIUM IN RELATION TO WOUNDING
IN THE BALSAM POPLAR.

BY

A. B. BROWN

Note: This paper has not yet been submitted for publication.

ACTIVITY OF THE VASCULAR CAMBIUM IN RELATION TO
WOUNDING IN THE BALSAM POPLAR.

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INTRODUCTION

For a long time it was thought, and apparently still is by many, that the vascular cambium plays an important rôle in the formation of callus tissue, but this belief derives no support at all from the recent work of Sharples and Gunnery (19), and of Sass (18). According to these investigators, callus tissue arises as the result of proliferation of medullary ray cells particularly, and not from the vascular cambium generally. However it is known definitely that the cambium responds to wounding, under certain conditions, to produce vascular elements.

Hartig (9) was the first to observe the basifugal development of cambial activity from the upper edge of a complete ring in woody shoots, and Sledge (20) has greatly increased our knowledge of this phenomenon in recent years. This upward spread is of limited extent, and is quite independent of the presence of growing buds and leaves, upon which the normal basipetal development of cambial activity in the shoot does depend. The present writer (2) has also observed the same

type of thing in ringed roots of poplar. Swarbrick (24) mentions an interesting case of local cambial activity as a result of wounding, in a completely disbudded apple shoot. In one of his experiments a small triangular piece of cortex and phloem was accidentally torn out, so as to leave the xylem exposed. Upon examination some time later, cambial activity was observed above, below, and lateral to the wound.

A major communication on the subject of tissue reorientations in woody plants, in response to wounding, has been contributed by Janse (10). He conducted an extensive series of ringing and wounding experiments, and appears to have been the only investigator to pay any attention to the behaviour obtaining below bridged rings and other types of wounds. The main value of his publication lies in his observations as such and the abundance of excellent photographs illustrating them, rather than in his interpretation of his observations. Apparently, his experiments were carried out during the growth period when normal cambial activity was proceeding, and he has nothing to say about local wound cambial activity independent of normal cambial activity. He showed, among other things, that when an oblique bridge of bark was left connecting the upper and lower edges of a ring, the cells within the bridge soon became reoriented to run in the same direction as the bridge, and that this change was a gradual one, becoming more and more marked in each successive layer of cells cut off by the cambium. A little later Teodoresco and Popesco (25) studied callus formation and the reaction of the cambium in relation to a ring in which a bridge of

bark in the form of a step (Czapek's ring) was left, connecting the upper with the lower edge. The wound was made when the cambium was active, and they found that ultimately the elements in the horizontal part of the bridge became reorientated to run transversely, with the result that uninterrupted vascular continuity between the parts above and below the wound was re-established. This type of behaviour is also discussed by Priestley (15) in relation to his theory of symplastic growth, and a detailed investigation of the changes involved in the reorientation of the cambium within the bridge has been carried out by Tupper-Carey (27). Somewhat similar results, although less detailed, are reported by Collins (4) who found that he could obtain zig-zag and spiral grain of the wood as a result of appropriate wounding.

This present investigation involves a critical study of the reaction of the vascular cambium to different types of wounds, made in shoots during the winter rest period. In this way it was possible to study local wound cambial activity for a considerable period of time before the normal basipetal flow of cambial activity from developing extension growth had reached the wound, and in the case of completely disbudded units, apart altogether from normal cambial activity. A number of significant correlations have revealed themselves, on the basis of which, the response of the vascular cambium to wounding is interpreted in terms of the interaction between a hormone present in the living bark, which is very probably identical with that promoting normal cambial activity as described recently by Snow (23), and a wound substance produced subsequent to wounding.

MATERIAL

The material used throughout this investigation was Populus balsamifera L., the balsam poplar. Main stems or leaders only were employed, and some 600 trees, yielding more than 1000 experimental units, have been involved in the work. The units practically always consisted of a length of shoot derived from the extension growth of one particular year, and varied in age from 1 to 4 years. Wounding was carried out during the winter months, December to May, when the cambium was dormant. The wounds were smeared with vaseline and the shoot placed vertically with its basal end immersed in water, where rooting took place very readily as the result of rapid development of preformed root initials. In all the experiments to follow the shoot was placed vertically, unless otherwise stated. It was found that by peeling off the bark from the shoots at the end of the experimental period, and allowing them to dry out, the new xylem formed locally subsequent to wounding showed up clearly upon the surface of the old wood. This procedure was used extensively throughout the investigation and proved to be of great value.

QUALITATIVE EXPERIMENTS

(a) Activity above and below a complete ring.

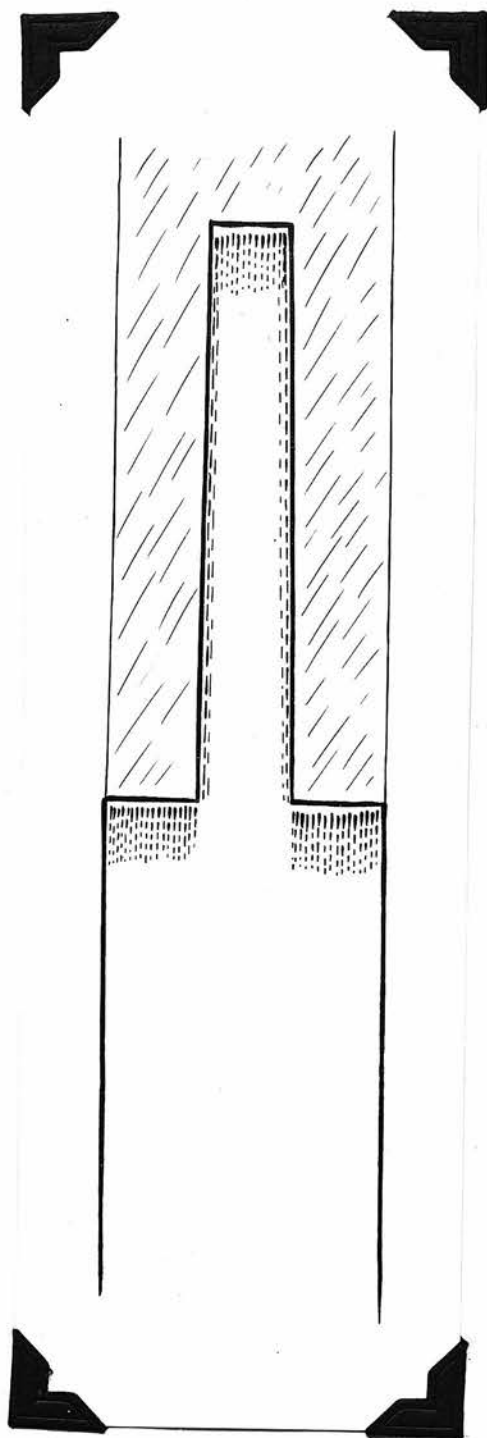
The following remarks apply to the local development of cambial activity above a complete ring, independent of normal cambial activity emanating from developing extension growth. In these experiments great care was taken to destroy the cambium completely, between the upper and lower edges of the ring. Cambial activity above a complete ring in balsam poplar is expressed by a very obvious basifugal gradient of xylem formation, spreading for a short distance distally from the upper edge of the ring. There is a marked tendency for this new xylem to be "piled up" just above the ring. In appearance this wood is not markedly atypical, at all events in transverse section. It consists of vessels, tracheids and well thickened fibres, with nests of parenchyma not infrequently included. The most obvious departure from typical stem wood is to be found in the shape of many of the vessel segments. They are narrow, and might readily be identified in longitudinal sections as tracheids with long tapering ends. On maceration of the wood however, these elements reveal themselves as vessel segments with very oblique end walls which are for the most part pitted, and the perforations between these vessel segments occur laterally at maturity, whatever their place of origin may have been morphologically.

The general belief is that no cambial activity originates from the lower edge of a complete ring, but this is certainly not the case in balsam poplar, where it was found that a very feeble basipetal

gradient of cambial activity does develop from the lower edge of a complete ring. Apparently the cambium cuts off cells mostly to the inside. In transverse section (Plate 2, fig. 1) these cells are more or less uniformly rectangular in shape, and they remain thin walled and unlignified with the exception of occasional small groups which lignify, without any appreciable expansion in any direction, to form scalariform and reticulately pitted tracheids. Vessels and fibres have not been found to occur. As many as 10 - 15 rows of cells, cut off to the inside in this manner by a well defined cambium, have been observed just below the lower edge of a complete ring. The gradient falls off rapidly, and usually within the space of a few millimetres ceases to be detectable. It has however been observed clearly in many cases for a distance of one centimetre from the lower edge of the ring. Relative to that obtaining just above a complete ring, the amount of cambial activity developing basipetally from the lower edge is indeed small. As far as the writer is aware this type of development has not been reported before. Sledge (20), in one case only, an internodal cutting of Sambucus, detected cambial activity at the distal end, where a few new elements had been cut off at one place, but apparently not completely around the shoot as in poplar.

A simple modification (text - fig. 1) of the complete ring yielded results of marked significance. In this case the ring was very wide, and a tongue of bark (cortex and phloem) was left, running distally from the lower edge. After some weeks the material was examined for cambial activity, with the result expressed by the dotted lines in the diagram. A feeble basipetal development of cambial

Text - fig. 1. Illustrating greater development of cambial activity longitudinally from the transverse margins than laterally from the longitudinal margins, in a wound where a longitudinal tongue of bark runs distally up the shoot, from what is really the lower edge of a complete ring.



Text - figure 1

activity was detected spreading downwards from the transverse margins, but extremely little laterally from the longitudinal edges of the wound. The elements formed were of the type already described as occurring below an ordinary complete ring, i.e. unignified with the exception of a few tracheids. A correlation that may be of some significance in this connection is that dying subsequent to wounding is always more extensive longitudinally from the transverse edges of the wound, than it is laterally from the longitudinal edges.

No evidence was obtained that cambial activity from the lower edge of a complete ring is dependent upon the development of adventitious buds, which occasionally arise from the callus tissue in this region. Such buds promote a local obliquely basipetal development of cambial activity in the shoot below them, but this activity is easily distinguished from the uniform development that is to be observed when no adventitious buds can be detected. Moreover there was no question of any stimulus to cambial activity passing across the ring, for precisely the same type of behaviour can be observed at the distal end of completely disbudded cuttings. The foregoing results are also of interest in comparison with callus formation in relation to ringing. Callus formation, just as cambial activity, is more marked at the upper edge of a complete ring than it is at the lower, where it does however usually occur to some extent.

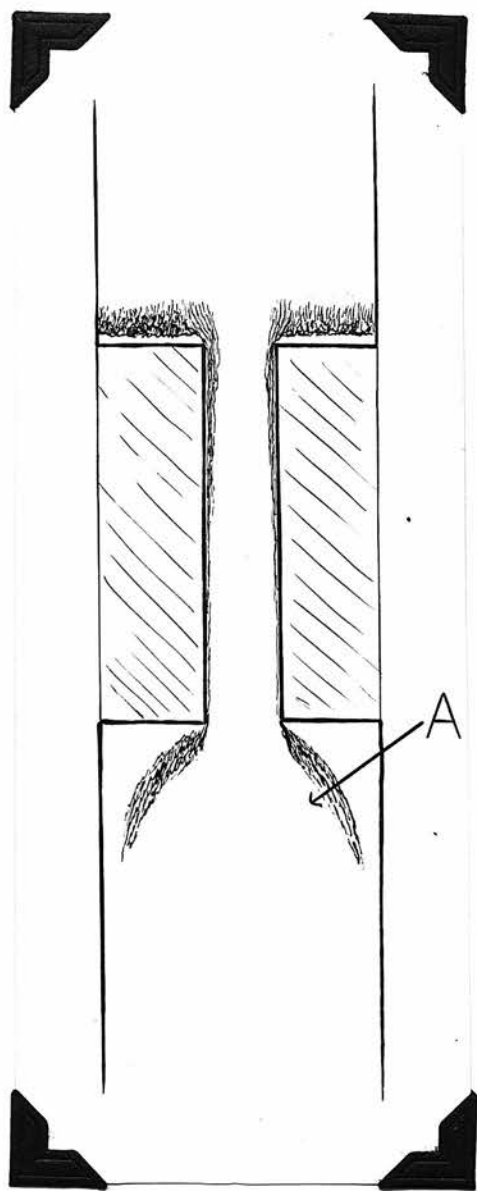
(b) The longitudinal bridge.

These observations apply to local cambial activity in relation to a wound where, instead of making a complete ring, a longitudinal bridge

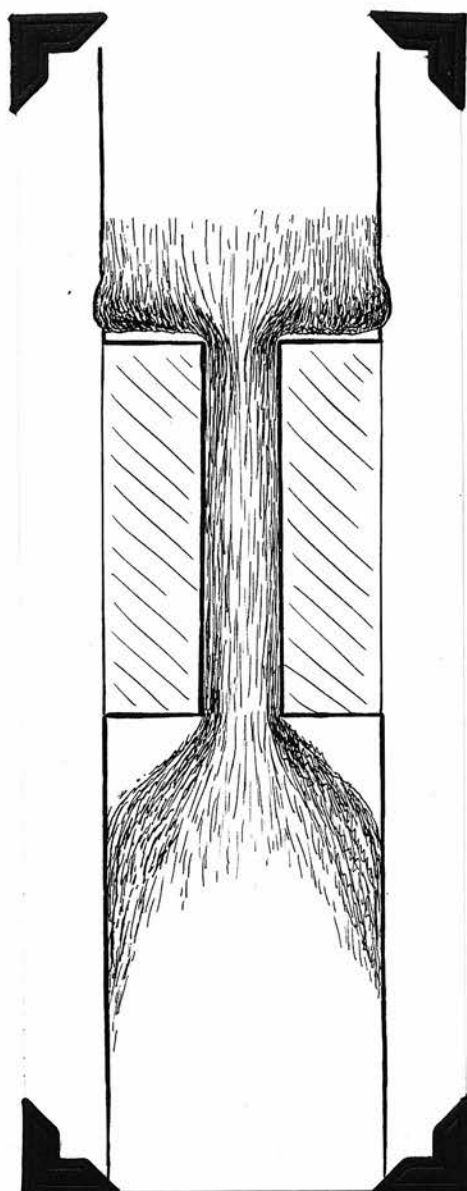
of bark is left, connecting the parts above and below the wound. In some shoots a complete ring was made some distance above the wound under consideration, and the shoot completely disbudded and lateral branches removed between the complete ring and the wound below. In others no complete ring was made above the wound, but the shoot was disbudded and laterals removed for a considerable distance above the wound. This was done in order to make it possible to observe local wound activity for some considerable time before the normal basipetal development of cambial activity from the developing extension growth reached that point. Actually however, the sequence of events was exactly the same in both groups. Text - figure 2 and figure 1 (Plate 1) illustrate the result obtained in relation to a wound of this type after 2 - 4 weeks. The arrangement depicted is visible to the naked eye when the bark is peeled off and the shoots allowed to dry out. It will be observed that just distal to the upper edge of the wound, a well defined basifugal gradient of xylem has been laid down, essentially similar to that obtaining above a complete ring. Within the bridge however cambial activity is basipetal, being undoubtedly more evident at the distal end of the bridge during the earlier stages of development. In transverse sections through the bridge it will be found that wedges of new wood, tapering off to the inside from the edges of the bridge, have been formed. Below the bridge cambial activity swings round on both sides to run obliquely down and around the shoot. The first signs of cambial activity are always to be observed just distal to the upper edge of the wound. Notice (text - fig. 2) that there is no cambial activity in the median

Text - fig. 2 Local cambial activity in relation to a longitudinally bridged ring, expressed in terms of xylem formation as revealed when the bark is peeled from the shoot.

Text - fig. 3 Same as text - fig. 2, but showing more extensive development.



Text figure 2



Text figure 3

line of the bridge itself, or in the shoot above and below in the same longitudinal line as the bridge. Particular attention should be paid to the fact that there is no cambial activity at the point A (which is definitely lateral to the longitudinal bridge), whereas cambial activity does occur lateral to it, not only at the same transverse level but also below that level. The obliquely basipetal spread is more extensive than the basifugal spread above the wound. However the depth of wood radially is always greater just above the upper edge of the wound than at any other point. Another especially interesting point is that there is definitely more cambial activity (including cell divisions, vessel formation and lignification) below the wound than within the bridge itself. This is very different from the state of affairs obtaining in relation to a similar wound, coming under the influence of the normal basipetal development of cambial activity for at least some considerable time. The present writer (2) has observed in aspen poplar, that in such a wound either in the shoot or the root, the width of the growth ring is always greater within the longitudinal bridge than it is above or below. Teodoresco and Popesco (25) also make a statement to the effect that the amount of new xylem and phloem formed within the zig-zag bridge in their experiments was abnormal, as if an attempt were being made to replace the tissues which had been cut away.

Development has been followed for as long as eight weeks, but no significant changes occur. There is a general increase in the amount of cambial activity (text - fig. 3). The wedges of xylem within the bridge ultimately meet, provided the bridge is not too wide, and again

depending upon the width of the bridge, the depth of wood may become more or less uniform across the bridge. Coincident with this, new xylem may make its appearance to a greater or less extent, above and below the wound in the same longitudinal line as the bridge. The obliquely basipetal development below the wound becomes more extensive, and swings round on both sides to meet behind (text - fig. 4). At the end of eight weeks, in those shoots with no complete ring above the wound, the normal basipetal development of cambial activity from the buds and leaves had usually reached the wound, but even then, in this material, there was still less cambial activity within the bridge, relative to that obtaining below the wound.

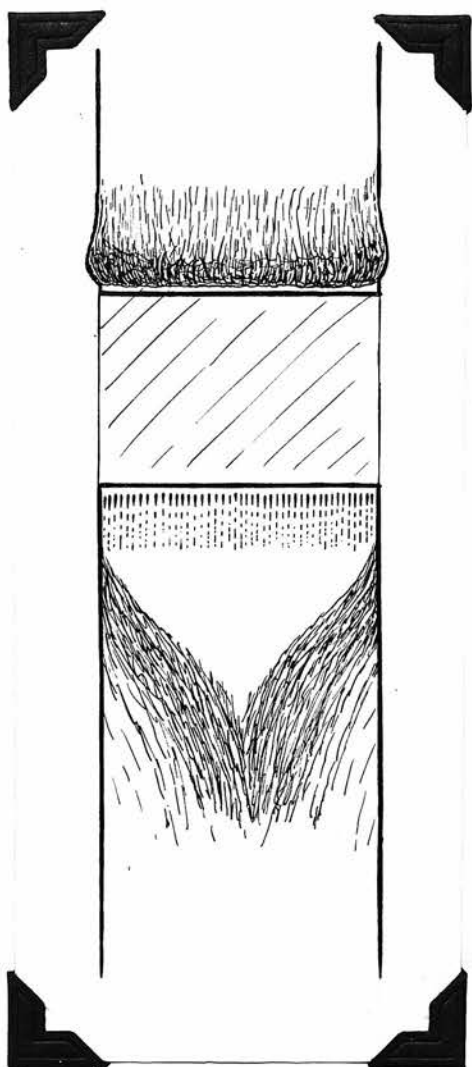
Text - figure 4 illustrates the state of affairs just below the wound, on that side of the shoot behind the longitudinal bridge. The obliquely basipetal development of xylem is shown swinging round the shoot from both sides and ultimately meeting. The dotted lines indicate the very feeble basipetal gradient of cambial activity, corresponding to that just below a complete ring, which can be detected in the region missed by the strong obliquely basipetal development. Within the bridge itself, the exceedingly feeble stimulation of cambial activity laterally from the margins, corresponding to that obtaining in a distally running tongue of bark as previously described (text - figure 1), is completely masked by the stronger basipetal development.

The xylem developing basifugally from the upper margin of the wound is essentially similar to that above a complete ring, consisting of vessels (mostly atypical and of the type already described), tracheids, fibres and included groups of parenchyma. Within the bridge

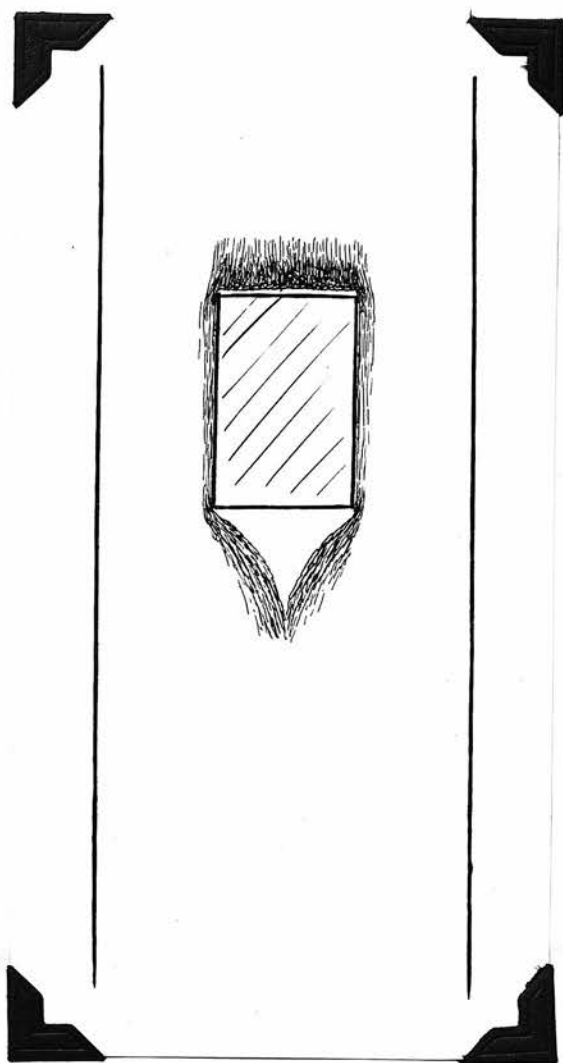
the new wood is usually more or less normal, except for the not infrequent presence of parenchyma. The obliquely basipetal development below the wound is however particularly interesting. Actually in that sheet of tissue the cells are all orientated, at least for a very considerable period of time, in the normal longitudinal direction. Undoubtedly development is obliquely basipetal, but it would not be so obvious to the naked eye perhaps, were it not for the peculiar mode of development of the vessels. In this new wood, vessels do not arise from the progressive vacuolation of vessel segments in longitudinal series, but rather from a series of cells running obliquely down and around the shoot. The result is that it is only the vessels as a whole that run obliquely basipetally, whereas the vessel segments themselves, the fibres, the unlignified parenchyma and the medullary rays practically retain their normal orientation (Plate 2, figure 2). The vessel segments are atypical however in that the end walls are definitely more oblique than usual, and the perforations between vessel segments tend to be lateral at maturity. Moreover the vessel segments are often shorter than those of the typical vessel. Even after as long as eight weeks, very little progress in the direction of a general reorientation of the tissue elements was observed in this material. A general reorientation is however complete within a much shorter period of time below a similar wound in the normally growing tree, during the season of active growth. These observations would seem to indicate that the process of vessel formation plays rather an important part in tissue orientations of the type just described.

Text - figure 4. Illustrating meeting behind the shoot of the obliquely basipetal development of xylem formation below a longitudinally bridged wound. The dotted lines indicate feeble cambial activity, similar to that occurring below a complete ring, in which there is no differentiation of vessels or fibres.

Text - figure 5. Local cambial activity in relation to a rectangular wound.



Text - figure 4



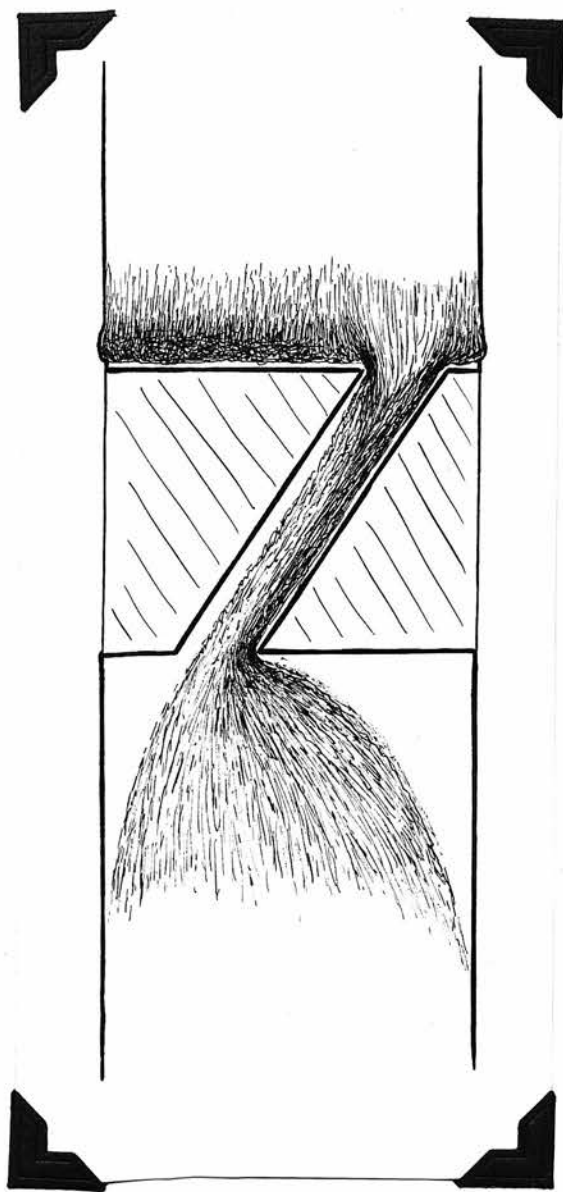
Text figure 5

Text - figure 5 illustrates cambial activity in relation to another wound, which is really a modification of the type just considered. In this case a rectangular piece of bark has been cut out. It is hoped that the drawing more or less explains itself, and nothing more will be said regarding it. An essentially similar arrangement is obtained around the wound resulting from the removal of a circular piece of bark.

(c) The oblique bridge.

A series of experiments was also set up, in which an oblique or spiral bridge of bark, instead of a longitudinal one, was left connecting the upper and lower edges of the wound (text - figure 6). It is proposed to allow the diagram to more or less tell its own story, which permits the following descriptive remarks to be brief. Here again a basifugal gradient of xylem is laid down from the upper edge of the wound. Development below the wound is obliquely basipetal, just as in the previous experiments. Within the bridge itself development is also obliquely basipetal, the grain of the wood running parallel to the edges of the bridge. The following significant points should be noted. Within the bridge cambial activity is most marked at the lower edge and decreases towards the upper. This is the more obvious the wider the bridge, but in a very narrow bridge it may not be detected at all. The lateral spread in the shoot below the wound is always more extensive on that side below the acute angle formed by the lower edge of the wound and the bridge. In the bridge itself reorientation of all the elements, including the medullary rays, to run in the

Text - figure 6. Local cambial activity in relation to an obliquely bridged ring, expressed in terms of xylem formation.



Text - figure 6

the same direction as the bridge takes place rather quickly, and may be complete at the end of five weeks. Below the bridge however, re-orientation is much slower, and at the end of eight weeks is still more or less confined to the vessels as a whole, the vessel segments as individual units and all the other elements still running practically longitudinally, in the same manner that obtains below the wound with a longitudinal bridge. A similar state of affairs is to be found in the bridge itself during the earlier stages of development (2 - 3 weeks). In sections through the bridge and transverse to the shoot, in material that has been developing for a longer period of time (5 - 8 weeks), it is found that the elements of the first formed xylem are all cut transversely, and that the later formed elements are cut progressively more and more obliquely until re-orientation is complete. Here again, the first expression of reorientation is to be found in the process of vessel formation from a number of segments in obliquely basipetal series. In contrast with the longitudinal bridge, it is not infrequently found that there is a greater radial width of new wood within the oblique bridge than occurs at any point in the shoot below it, particularly if the bridge is rather narrow.

(d) The zig-zag bridge (Czapek's ring).

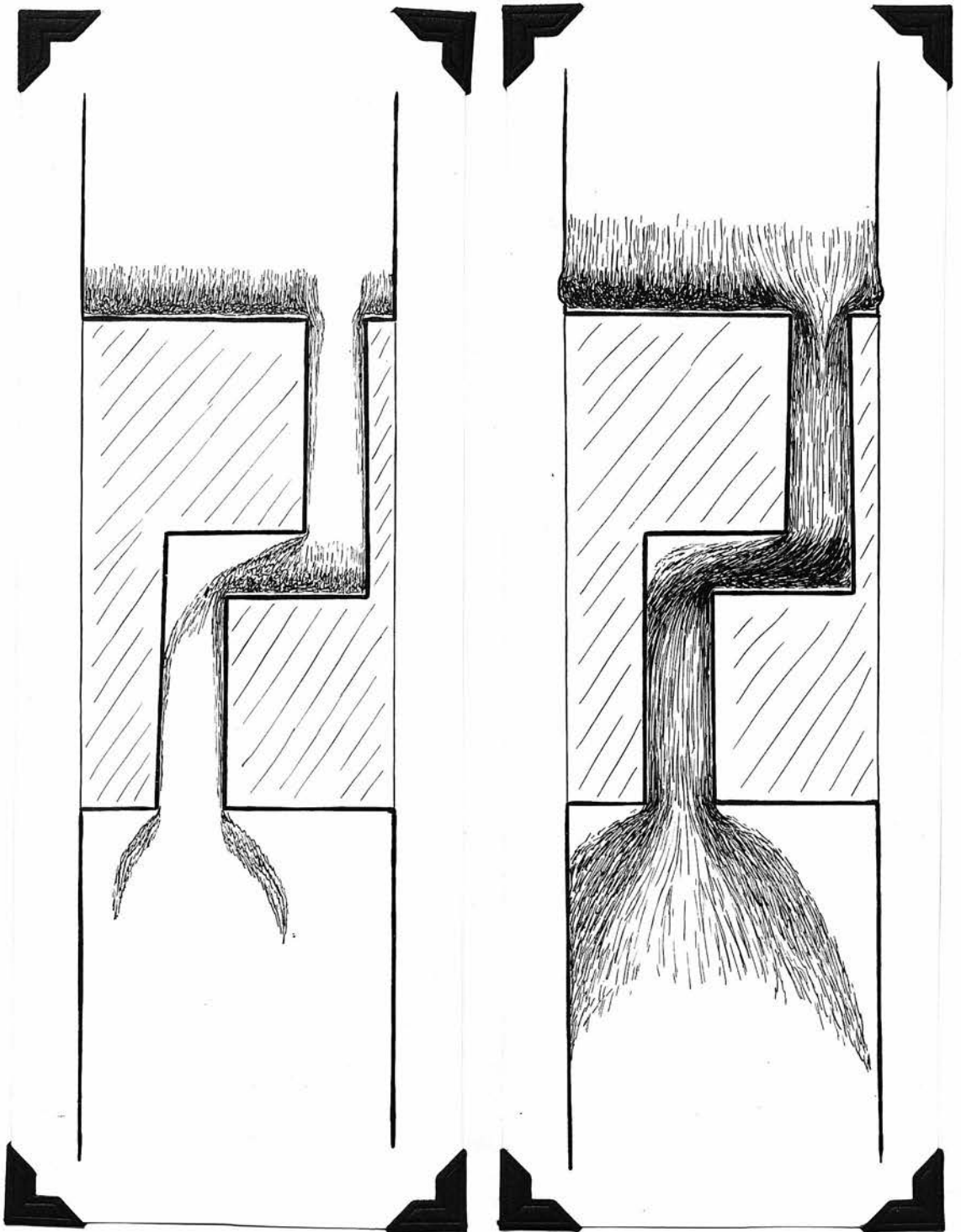
Development at an early stage, 10 - 14 days, in relation to this type of wound is depicted in text - figure 7. The first signs of cambial activity may be observed about the same time, just above the upper margin of the wound and in the horizontal part of the bridge, exclusive

of that area which is common to the lower vertical strip. Within the upper vertical strip cambial activity is basipetal, and essentially similar to that in the simple longitudinal bridge. Notice that an obliquely basipetal development, from the base of the upper vertical part of the bridge into the horizontal portion, is shown. This may not be obvious if the bridge is very narrow. In the lower vertical strip, cambial activity is for the most part basipetal, but towards the distal end, there is also a definite obliquely basipetal spread across the bridge, from the horizontal strip to the farther margin of the vertical strip. Below the wound the obliquely basipetal development is often rather more marked on one side, viz., that side directly below the horizontal portion of the bridge. Otherwise the situation below the wound is exactly the same as that obtaining below the simple longitudinal bridge, and again it is usual to find more cambial activity below the wound than occurs within the lower vertical portion of the bridge, at all events towards its basal end.

A later stage in development, after 4 - 5 weeks, is shown in text - figure 8. There is a marked "piling up" of new xylem, not only above the upper margin of the wound, but also in the horizontal part of the bridge. Usually there is no appreciable difference in the amount of cambial activity in the upper and lower vertical strips. Provided the bridge is fairly narrow, the obliquely basipetal development below the wound may ultimately be practically equal on both sides, but if the bridge is sufficiently wide the greater spread on one side may be maintained.

Text - figure 7. Local cambial activity in relation to Czappek's ring (zig-zag bridge) expressed in terms of xylem formation.

Text - figure 8. Same as text - figure 7, but showing more extensive development.



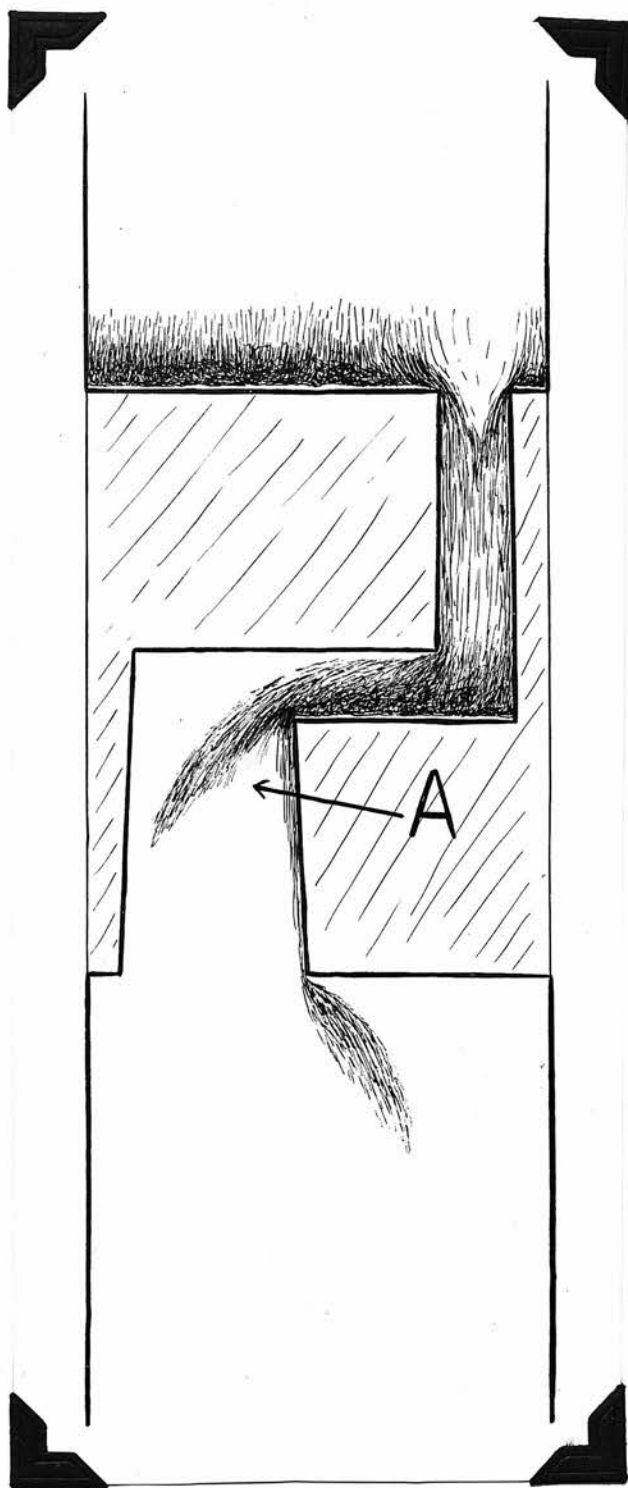
Text - figure 7

Text - figure 8

Text - figure 9 shows a simple modification of the zig-zag bridge, in which the lower vertical strip is very wide. In such a wound the obliquely basipetal spread of cambial activity from the horizontal strip across the lower vertical strip, and the basipetal spread down the nearer longitudinal margin stand out as two distinct developments, diverging from one another so as to leave an area (A), in which there is no cambial activity. The diagram was drawn from a specimen, three weeks after the beginning of the experiment. After a longer period of time, the obliquely basipetal development may ultimately reach the farther margin of the lower vertical strip and travel basipetally down it, just as in the narrower zig-zag bridge depicted in text - figure 7. The phenomenon just described is of great significance and will be enlarged upon later, but it might be worth while, at this point, to compare it with behaviour in relation to a wound previously described (text - figure 5), where a rectangular piece of bark has been cut away. In this latter case development is basipetal down the longitudinal margins of the wound, and there is no obliquely basipetal spread corresponding to that obtained in the wide lower vertical strip of the modified zig-zag bridge.

The structural characteristics of the basifugal development of xylem above the wound, of the basipetal development in the two vertical strips, and of the obliquely basipetal spread below the wound are exactly similar to those obtaining in the same relative positions in the wound with the longitudinal bridge of bark, and no further comment is called for in this connection. Within the horizontal strip of the bridge, all the elements of the first formed xylem retain their normal

Text - figure 9. Local cambial activity in relation to a simple modification of Czapek's ring, in which the lower vertical strip of the zig-zag bridge is made quite wide.



Text - figure 9

orientation in the longitudinal direction, but the vessels arise from a number of segments in lateral or obliquely lateral series. As development proceeds the situation becomes quite complex within the horizontal strip, involving much twisting and turning of the elements, and resulting in a very complicated grain of the wood. Even after as long as eight weeks, complete and uniform reorientation of the elements in the transverse direction was not observed, in this material, but it does occur ultimately in a similar wound in the actively growing tree.

Tupper-Carey (27) has followed in detail the structural changes involved in reorientation of the tissues within the bridge in Czapek's ring. Her conclusions need not be discussed here, but she does not stress at all the process of vessel formation as an important factor in this reorientation. In view of the observations, made in this present investigation, on the process of vessel formation from a number of cells in obliquely basipetal series (Plate 2, figure 2), within the oblique bridge and below all three bridged wounds, the writer is strongly inclined to attribute to this process an important rôle in the ultimate reorientation of the tissues as a whole, and is also of the opinion that the details might be studied more conveniently, and certainly with less complications, in the oblique bridge than in the zig-zag bridge. These observations lend some measure of support to a former suggestion by the writer (1), that reorientation of the tissues in the form of a flow-pattern, below the sucker bud in poplar roots, maybe linked up with the process of vessel formation.

QUANTITATIVE EXPERIMENTS

A large number of shoots were treated as follows. Towards the distal end a complete ring was made, the shoot completely disbudded and lateral branches removed below the ring. Some distance below the ring, for example two inches, a definite wound was made, and then another complete ring at, let us say, four inches below that wound. Finally another wound, exactly similar in shape and size to that between the two complete rings, was made at a much longer distance, for example ten inches, below the second complete ring. The shoot was cut through four inches below this wound and then set up vertically with its basal end immersed in about one inch of water. The definite wounds referred to might be of any type. Actually the circular wound made by means of a sharp cork borer, and bridged rings were used extensively. The exposed xylem was thoroughly scraped to ensure destruction of the cambium, and the wounds were vaselined in the usual way. The distances between the wounds and the complete rings above varied according to the material, but the situation was always this, that the shoot bore two similar wounds, one of which had a short length of bark distal to it, and the other a much longer length of bark distal to it. In some the shorter unit was distal on the shoot, and in others the longer. On examination after 4 - 5 weeks it was invariably found that there was more cambial activity (new xylem) around the wound with the greater length of bark distal to it.

The same relationship between the amount of cambial activity around a wound and the amount of bark distal to the wound, was also

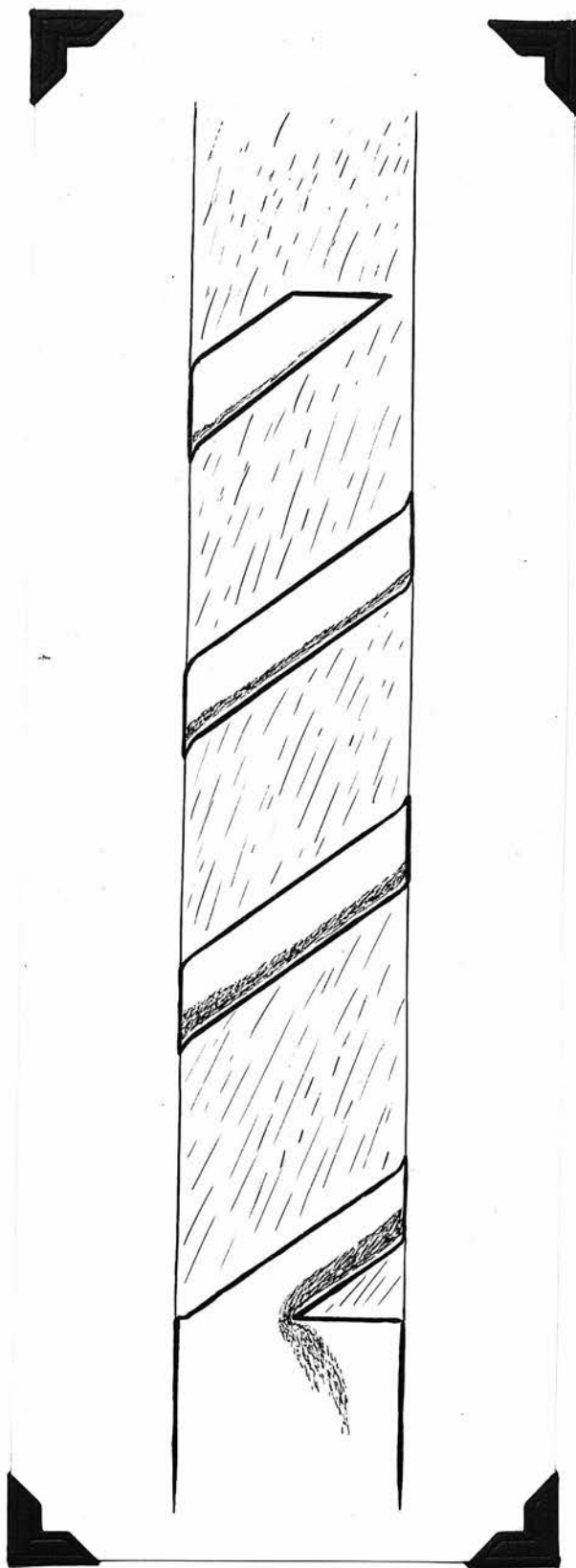
expressed clearly and invariably in another type of experiment (Plate 1, figure 1). In the particular experiment illustrated the arrangement was as follows. Unit C had a complete cylinder of bark, seven inches in length, distal to the longitudinal bridge. Unit B had the same length of bark distal to the bridge, but the area of bark was much reduced by removal of about one-half of the cylinder, and in the third unit A, about three-fourths of the cylinder of bark was removed, so that only about one-fourth remained in the form of a long narrow strip seven inches in length. The three units were all located on the same shoot in three-year-old wood, and within the limits of a single year's extension growth. Unit A was the most distally situated on the shoot, and unit C was distal to unit B. In other similar experiments, the relative positions of these units to one another were varied. The result of such an experiment is expressed clearly in the photograph¹ (Plate 1, figure 1); which shows the extent of xylem formation, as revealed when the bark was stripped off after four and a half weeks. Here again it is evident that the more bark there is distal to a wound, the greater is the extent of development of cambial activity in relation to the wound. It does not follow that the width of the transverse cut as such influences in any way the amount of cambial activity, but it does indicate lateral transportation of the stimulus to cambial activity.

¹ Much detail will be observed if the reader cares to examine this photograph with a hand lens. No doubt this detail will be lost in the published plate.

Still another experiment, leading to the same conclusions with regard to the proportional relationship between wound cambial activity and the amount of bark distal to the wound, and also to lateral translocation of the stimulus to cambial activity, is depicted in text - figure 10. Here, a spiral tongue of bark was left running distally from the lower margin of a very wide complete ring. It was observed that xylem formation increased gradually towards the base of the spiral tongue.

Experiments were also performed to determine the effect, if any, of developing buds and leaves. Main shoots of the same age were selected in pairs, the members of each pair being as uniform in all respects as could be determined by external examination. In one of each pair, a definite wound was made, and the shoot completely ringed some considerable distance, at least twelve inches, distal to the wound. The unit was completely disbudded below the complete ring. The other member of the pair was cut so that approximately the same amount of bark was left distal to the wound, but a few buds, usually two or three, were allowed to remain and develop towards the distal end. Only the distally situated buds were left, because it was desired to observe their effect upon wound cambial activity, before the normal basipetal gradient of cambial activity from the developing buds and leaves had reached the level of the wound. The experimental units were therefore of two types, those bearing buds and those without buds. The shoots composing the latter did of course bear buds above the complete ring distal to the wound, but these buds were without the experimental unit as such. Many types of wound were employed, but for microscopic

Text - figure 10. Cambial activity, expressed in terms of xylem formation, in relation to a spiral tongue of bark running distally up the shoot from a transverse margin.



Text - figure 10

examination the longitudinally bridged wound is perhaps the most convenient.

The leaves began to emerge from the buds after about two weeks, and the material was examined at intervals later. In this way definite evidence was obtained, in several series of experiments, that the presence of developing buds and leaves within the unit increased the amount of cambial activity in relation to the wound, and this effect was observed undoubtedly before the normal basipetal gradient of cambial activity emanating from the developing extension growth had reached the wound. The clearest results were obtained four to six weeks after the beginning of the experiment, i.e. two to four weeks after emergence of the leaves from the buds. Not only was there a definite increase in the amount of cambial activity around the wound, but lignification of the new xylem was also more marked. There was also a tendency, not invariable however, for the vessels to be wider when developing buds and leaves were present on the unit. The above effects were not observed until after the buds had opened and the leaves had developed to a considerable extent, which would indicate that the increase in wound cambial activity is to be related not simply to the presence of buds within the unit, but rather to the development of leaves from these buds.

The following table showing the number of vessels at certain points in relation to a wound, in fourteen pairs of contrasting units, with and without developing buds and leaves, gives some idea of the results obtained. In the first four pairs, a small rectangular piece of bark was cut out on March 30, the length of bark distal to the wound was twelve inches in all cases, and the experiment ran until May 2. The number of vessels that had developed down the longi-

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tudinal margins of the wounds was counted from transverse sections.

In the next six pairs of units, the wound employed was Czapek's ring, there were twelve inches of bark above the wound in all cases, and the experiment ran from March 30 until May 3. The figures given are the numbers of vessels in the lower vertical strip of the bridge. In the last four pairs, a circular wound was employed, there were twenty inches of bark distal to the wounds, and the experiment ran from April 6 until May 7. The vessels were counted in sections cut through the diameter of the wound and transverse to the unit.

Table

Numbers of vessels in contrasting pairs of units, with and without developing buds and leaves.

<u>Without buds</u>	<u>With buds</u>
42	87
40	76
25	98
26	53
69	104
80	144
28	65
154	234
67	113
88	217
101	265
173	264
178	324
231	327

In none of the material considered in the foregoing table had the normal basipetal gradient of cambial activity reached the wound, in units bearing developing buds and leaves.

The effect of developing buds and leaves is illustrated quite typically, in two photomicrographs (Plate 2, figures 3 and 4) of sections from a contrasting pair of units in another experiment. In this case the wound employed was the longitudinally bridged ring. There were nineteen inches of bark distal to the wound in both units, and the experiment ran from April 18 until May 27. The sections photographed were obtained from corresponding positions within the longitudinal bridge in both units. Figure 3 (Plate 2) shows the extent of development in the unit in which the two most distally situated buds were allowed to develop, and figure 4 illustrates the corresponding development in the completely disbudded unit. In this particular case the normal basipetal gradient of cambial activity faded out about three inches above the wound, in the unit bearing developing extension growth.

Sledge (20) reports that he found no difference in the amount of cambial activity at the base of disbudded cuttings relative to control cuttings, after a period of one month. The present writer found the complete ring a very unsatisfactory type of wound to use in quantitative experiments, on account of the "piling up" of cambial activity above the ring, which made it extremely difficult to detect any but the most obvious differences in the extent of development, even when the material was examined microscopically. A similar "piling up" of cambial activity also takes place at the base of woody cuttings, where the conditions are essentially similar to those above a complete ring.

THE EFFECT OF GRAVITY UPON WOUND CAMBIAL ACTIVITY.

Experimental units were completely ringed towards the distal end, and all buds and laterals below the ring removed. Some considerable distance, at least twelve inches, below this ring a modified ring was made in which two longitudinal bridges on opposite sides of the shoot were left, connecting the proximal and distal margins. Another complete ring was then made about six inches below this wound, and the shoot cut through approximately six inches below this second complete ring. In other units, two circular wounds on opposite sides of the shoot were often employed instead of the doubly bridged ring. The shoots were arranged practically horizontally with their basal end in water in a very simple manner, viz., by inserting them into ordinary cylindrical metal cans, about five inches in diameter and with an inwardly projecting rim, so that the cut end lay under the rim, and the shoot rested upon the opposite edge. If the cans were filled with water, two shoots pointing in opposite directions could be conveniently inserted in this manner, and a very stable arrangement resulted. The units were arranged so that the longitudinal bridges, or the circular wounds, lay one directly above the other, on opposite sides of the shoot.

After about one month, material that had been kept in complete darkness during the period of the experiment, was examined for local wound cambial activity, when a marked response to gravity was clearly revealed. There was always distinctly more cambial activity on the upper than on the lower side. This response was evident not only

in relation to the doubly bridged wound and the circular wounds, but also just distal to the lower complete ring. It was also observed that in many cases, the preformed root initials on the upper side had undergone marked swelling, where they occurred along practically the full length of the unit, whereas no such activity was evident on the lower side. Figure 2 (Plate 1) shows clearly, in longitudinal and transverse section, the marked difference in the amount of new xylem on the upper and lower side, immediately distal to the lower complete ring in two three - year - old units which had been developing in the horizontal position from May 13 until June 10, in complete darkness. The same marked response was obtained in material developing horizontally in diffuse daylight where the upper side received more light than the lower, but the response is clearly one to gravity and not to light. Actually a number of experiments, which need not be described here, were performed to test the action of diffuse daylight, and no evidence of a light effect on the amount of wound cambial activity was obtained. The effect of gravity was still quite marked in units lying at an angle of 45° to the horizontal.

In another experiment, horizontally placed shoots were rotated through an angle of 180° once every twenty-four hours for one month, and at the end of that time no difference in the amount of wound cambial activity on opposite sides of the unit could be detected.

Similar experiments were also set up to determine the effect of gravity on the normal basipetal development of cambial activity emanating from growing buds and leaves. In this case, lengths of main shoot were trimmed so that only the more distal buds remained. They

were placed horizontally with their basal end in water in the manner previously described, and examined about two months later, when it was found that there was distinctly more cambial development on the upper than on the lower side. Priestley and Tong (16) obtained the same response to gravity in vertical shoots of Acer and Fraxinus, after they had caused them to develop in the horizontal position for some time. Apparently therefore normal cambial activity and local wound cambial activity in balsam poplar respond similarly to the force of gravity. No difference in the degree of lignification of the xylem on the upper and lower sides in horizontally placed units was detected, either with respect to normal cambial activity or to local wound cambial activity.

It will be recalled that a very feeble basipetal gradient of cambial activity, in which vessels and fibres do not occur, arises from the lower margin of a complete ring, but no difference in the extent of development on the upper and lower side in horizontally placed units could be detected.

DISCUSSION AND INTERPRETATION OF RESULTS.

(a) Quantitative results.

Snow (23) has very recently succeeded in promoting apparently normal cambial activity in shoots by means of pure auxin-a and hetero-auxin (B indolyl acetic acid), thus confirming his earlier conclusion (22), that the stimulus emanating from the developing leaves to activate the cambium in the stem below is of the nature of a hormone. In the opinion of the present writer, hormone action appears to supply the most reasonable explanation of the quantitative results obtained in the foregoing experiments on cambial activity in relation to wounding. This suggestion is based upon the following considerations. A definite quantitative relationship has been established between the amount of cambial activity in the vicinity of a wound and the amount of bark distal to the wound. This result would be readily understood in terms of the downward movement of a hormone present in the bark. It has also been shown that wound cambial activity is further promoted by the presence of developing leaves distal to the wound, an effect which can be detected before the normal basipetal development of cambial activity emanating from these leaves has reached the wound. This indicates that developing leaves are a source of the hormone promoting wound cambial activity, which suggests further that this hormone is probably the same as that emanating from leaves to promote normal cambial activity. In terms of this suggestion, it is implied that the hormone moves to some extent in advance of the normal basipetal development of cambial activity, which is indeed not at all unlikely. That

the hormones promoting normal cambial activity and wound cambial activity are probably one and the same is also supported by the response of these two processes to the influence of gravity. In both cases, development is greater on the upper than on the lower side. Finally, cambial activity in relation to wounding, apart from that obtaining at the lower edge of a complete ring, is not so very different from normal cambial activity. The same types of elements are produced in both cases, at all events so far as the xylem is concerned, and presumably in the phloem also. If a hormone is involved in normal cambial activity, it would therefore appear reasonable to suspect hormone action in relation to wound cambial activity, and any evidence in that direction assumes significance.

In order to explain the response of both normal and wound cambial activity to gravity, it would appear to be necessary to postulate accumulation of the hormone on the upper side of horizontally placed shoots. There arises in this connection, quite independently of wound cambial activity, a very interesting problem which has not hitherto been stressed. Snow (23) has indicated that cambial activity and cell extension in stems are probably promoted, under natural conditions, by the same growth hormone, viz., auxin-a. According to the well known Cholodny-Went theory of geotropism, the growth hormone accumulates on the lower side of plants placed in the horizontal position, and this has indeed been shown to be true experimentally. On the other hand the cambial hormone, which according to Snow, is in all probability identical with the growth hormone,

must apparently accumulate on the upper side of woody dicotyledons when they are placed horizontally. Moreover the new extension growth of woody dicotyledons is definitely negatively geotropic, so that if the cambial hormone, and the growth hormone promoting cell extension and geotropic curvature are identical, we are forced to conceive of the hormone accumulating on the lower side in the region of negatively geotropic curvature, in terms of the Cholodny-Went hypothesis, and then on the upper side in older portions of the shoot, in order to explain the observed relationship between cambial activity and gravity. This is but one of many paradoxes to be found in the field of plant hormones, which are however well worth stressing, if only in order to offset any tendency towards acceptance of an as yet unwarranted simple relationship between plant hormones and the processes with which they are connected.

Sledge (20) has suggested that a wound stimulus, functioning through the injection with sap of the intercellular spaces in the vicinity of the cambium, plays an important part in the initiation of basifugal cambial activity at the base of woody cuttings. He then goes on to say that the absence of cambial activity at the distal end of cuttings, where presumably the same conditions of sap injection must hold, and which cannot always be accounted for by a basipetal transport of food, points to the existence of another "unknown factor" which is also involved. It has been shown in the present investigation that a very limited amount of basipetal cambial activity does arise from the lower edge of a complete ring in balsam poplar. However it would still appear to be necessary to post-

ulate Sledge's "unknown factor" in order to explain, not only the great difference in the amount of cambial activity just above and below a complete ring in completely disbudded units, but in addition the marked contrast in differentiation subsequent to cell division in the two cases. In terms of the suggested explanation of the quantitative results obtained in this present investigation, the "unknown factor" is now interpreted as being a hormone.

The question of food supply as a possible alternative to the hormone hypothesis calls for a certain amount of discussion. Sledge (20) came to the conclusion that polarisation of wound cambial activity in woody cuttings could not be accounted for as the result of a basipetal movement of food material. The same opinion with regard to normal cambial activity is shared by Snow (21, 22, 23) and others. Loomis (12) found that the relationship between active leaves and cambial activity in the shoot below could not be correlated with the production of carbohydrates by the leaves, since carbohydrate levels were higher in sprouting segments which showed but little cambial activity, than in leafy segments where cambial activity was marked. Then again, Priestley and Tong (16) found that, although there was definitely more starch on the upper side of horizontal branches of Tilia and Acer showing marked epitrophy (i.e. greater radial growth on the upper relative to the lower side), in Crataegus which also shows marked epitrophy of its horizontal branches, starch was deposited more heavily on the lower side. In this present investigation no special effort was made to follow the movement and distribution of starch, but it was observed that there

was definitely less starch in units bearing developing buds and leaves than in contrasting units which had been disbudded, whereas wound cambial activity was more marked in the former. It is of interest in this connection to note that Loomis (12) has shown that during the earlier development of leaves and sprouts from buds, the movement of food materials tends to be upwards. On the whole therefore, it would appear that a more satisfactory explanation of the quantitative results obtained in this work is to be found in terms of hormone action, rather than food supply.

The marked swelling of preformed root initials on the upper side only of horizontally placed units of balsam poplar is of more than passing interest. It has been shown by Thimann and Koepfli (26) and also by Cooper (5), that root formation is promoted by the same growth hormones that bring about cell extension in stems, and Laibach (11) reports that heteroauxin promotes both the development of roots and callus tissue in stems. In other words the same growth hormones promote cell extension, root formation and cambial activity, as well as a number of other processes which need not be detailed here. We have therefore the interesting correlation between stimulation of root initials, and the greater development of wound cambial activity on the upper side of horizontally placed shoots, which does in a measure support the writer's suggestion, that the hormone promoting normal cambial activity is also involved in the development of local wound cambial activity.

(b) Qualitative results.

It will probably be admitted that the observed behaviour of the cambium in relation to wounding cannot be explained completely satisfactorily, simply in terms of the accumulation or concentration of a hormone moving basipetally down the shoot. Consider for example the longitudinally bridged wound (text - figure 2 and 3, pg. 12). In this case the hormone will tend to accumulate above the upper transverse margin, but the experiment illustrated in figure 1 (Plate 1) indicates that lateral translocation takes place quite readily. One would therefore expect to find a greater concentration of hormone within the bridge than just below it. Nevertheless it has been shown definitely that there is more cambial activity in the obliquely basipetal development below the wound, than occurs within the bridge itself. In other words, the amount of cambial activity is not wholly determined by the concentration of hormone. Then again, consider the absence of cambial activity in the region A (text - figures 2, pg. 12 and 9, pg. 27), and the occurrence of cambial activity lateral to it, where presumably the concentration of hormone is lower. Apparently therefore some other factor plays a part, something no doubt in the nature of a wound stimulus.

At this point the author would like to stress particularly the fact that extreme care was taken in the making of wounds, in relation to which, critical observations of the type to be discussed in this section were obtained. The importance pertaining to discontinuity of the elements of the bark, as a result of transverse incisions was early recognised, and special care was taken to avoid cutting into the bridge

itself, particularly at the basal end. Moreover a common practice employed, which is indicated in many of the text - figures, was to make longitudinal bridges and the like, slightly wider at the basal end than at the distal end. In a word, the writer is satisfied that the observations to be discussed now are truly significant and not the result of imperfect technique, which as a matter of fact is entirely precluded in certain developmental relationships, showing definite homologies with other cases into which the question of technique might conceivably enter.

The conception of a wound stimulus in relation to wound cambial activity is not without precedent. Sledge (20) postulates a wound stimulus in his work, and conceives of it as functioning through the injection with sap of the intercellular spaces in the vicinity of the wound. Such an explanation is not however, in the opinion of the present writer, sufficient to explain the observed behaviour. It would imply less sap injection within the bridge than below it, which might be possible, but it would not explain the absence of cambial activity at the point A (text - figure 2, pg. 12), where presumably the same conditions of sap injection must exist as in the region lateral to it, where cambial activity does occur. Similarly it could not explain the behaviour depicted in text - figure 9, pg. 27.

Only one attempt has ever been made hitherto to explain development below bridged wounds and the like, and it is to be found in the work of Janse (10). This investigator was interested primarily in the following question. Does there exist in the plant, a force or ac-

tion which has the tendency to drive or push substances always and invariably in a given direction, ordinarily downwards? As the result of a series of wounding experiments, he came to the conclusion that there was such a force, and he called it the "force spéciale", a term which he ultimately replaces by "impulsion basipétale". He observed the obliquely basipetal development of cambial activity below bridged wounds, and he interpreted this phenomenon as the result of the action of two other forces, viz. the "attraction du cambium" and the "attraction des tissus traumatiques", upon his third force, the "force spéciale" or "impulsion basipétale". Let us now consider a particular case, for example, development below a rectangular wound of the type depicted in text - figure 5 pg. 17. Janse's interpretation would be as follows. Below the lower transverse margin of the wound, two forces are to be considered, (1) the "attraction du cambium" which acts in a horizontal direction, simply because the margin of the wound is transverse (if the wound margin were oblique the direction of this force would also be oblique, according to Janse), and (2) the "attraction des tissus traumatiques" which acts perpendicularly to the wound margin. These two forces set up, in the region below the wound, a so called field of attraction which reacts upon the "impulsion basipétale" acting longitudinally from above downwards, in such a way as to cause lateral diversion of the "impulsion basipétale" below the wound. As a result of this, food material is also diverted laterally in this region to nourish the cambium in its path. He does however write as follows: "Il faut se rappeler cependant ici que l'afflux de nourriture ne doit être considéré comme la cause du dévelop-

pment des tissus, mais que c'est au contraire la division des cellules et leur agrandissement qui sont les causes du courant qui va se diriger vers elles". But nevertheless, throughout his paper, he clearly considers food supply to be the important factor in development subsequent to the change in direction of the "impulsion basipétale"

Janse's interpretation is decidedly obscure, if only by dint of lack of definition of his forces. His "impulsion basipétale" may be interpreted as being simply the recognised tendency for cambial activity to develop normally in the basipetal direction, and it is generally conceded that the direction may be obliquely basipetal, without necessarily postulating the action of contributory forces. He stresses greatly the action of the "impulsion basipétale" upon the downward movement of food materials, whereas the same reaction of the cambium can be observed in relation to wounds in regions where the general movement of food materials is upwards, e.g., in regions bearing developing buds or sprouts (c.f. Loomis 12). Then again, it is by no means clear how Janse would explain the absence of cambial activity in the region A (text - figures 2, pg. 12 and 9, pg. 27), in terms of the interaction of his three forces.

The present writer is also of the opinion that a wound stimulus is involved in cambial activity in relation to wounding, and ventures to suggest that a satisfactory explanation is to be found in the conception of this wound stimulus in terms of a definite wound substance. This hypothesis is based upon the observations made in this investigation, and upon certain very significant correlations. It will first of all be stated briefly. The main points are as follows:

- (1) Subsequent to wounding a definite wound substance is produced as a result of death or dying of the cells bordering upon the wound.
- (2) The amount of this wound substance produced is proportional to the extent of dying.
- (3) The wound substance can of itself promote cell division only in the cambial layers.
- (4) Differentiation of more or less typical xylem and phloem, and in addition further cell division, is promoted by the cambial hormone which moves basipetally down the living bark and reacts with the wound substance to give the observed behaviour.

In the discussion to follow the writer proposes at times to discuss his observations in terms of the hypothesis, and at other times the hypothesis will be discussed in terms of the observations and correlations which led to its formulation. In terms of the hypothesis, the marked contrast in local cambial activity at the upper and lower margins of a complete ring is readily understandable. Development at the lower margin is to be attributed almost solely to the action of the wound substance, causing cell division only in the cambial layers. The absence of differentiation (with the exception of a few tracheids) to form vessels and fibres is the result of depletion in this region of the cambial hormone, on account of its basipetal transport down the shoot. Cambial activity involving differentiation of xylem just above a complete ring is the result of interaction between the wound substance and the cambial hormone which moves basipetally towards the wound margin from above. However the difference between cambial activi-

ty at the upper and lower margins of a complete ring is not simply the presence of vessels and fibres in the one case, and their absence in the other. It is clear that the cambium divides more frequently above a complete ring than below it, so that one has to conceive of the cambial hormone as promoting not only differentiation of xylem elements, but also, as might indeed be expected, further cell division.

Consider now, behaviour in relation to the type of wound depicted in text - figure 1 pg. 8, in which a longitudinal tongue of bark runs distally from the lower margin of a complete ring. Here again there is little or no differentiation of vessels and fibres, on account of basipetal transport of the cambial hormone. The interesting points are that stimulation of cambial activity is greater longitudinally from the transverse margins than laterally from the longitudinal margins, and the exceedingly suggestive correlation that dying of the cells subsequent to wounding is also more extensive longitudinally from the transverse margins than laterally from the longitudinal margins. Hence the hypothesis to the effect that production of wound substance is proportional to dying subsequent to wounding.

It has been shown that there is definitely more cambial activity above and below a longitudinally bridged ring than occurs within the bridge itself (text - figures 2, and 3, pg. 12). The correlation that immediately suggests itself here, is that dying subsequent to wounding is more extensive longitudinally from the upper and lower transverse margins of the wound than laterally from the longitudinal margins, which implies that there will be more wound substance pro-

duced just above and below the wound than within the longitudinal bridge. On this basis therefore, it is suggested that the observed behaviour might be interpreted as the result of a low concentration of wound substance within the bridge acting as a limiting factor, with the proviso that the limiting action is relative, not absolute. Precisely the same interpretation can be applied to behaviour in relation to Czapek's ring (text - figures 7 and 8 pg. 24), where again there is more cambial activity in the obliquely basipetal development below the wound than occurs within the vertical strips of the zig-zag bridge. On the other hand, the condition just discussed is not apparent in relation to the obliquely bridged ring, which is just what might be expected, since dying longitudinally from a definitely oblique wound margin is of the same order as that obtaining from a transverse margin. One would not therefore expect to find wound substance limiting within the oblique bridge.

It still remains to explain a peculiar feature of the obliquely basipetal development below bridged wounds and the like. Let us consider specifically the longitudinally bridged wound (text - figure 2, pg. 12), although behaviour below all bridged wounds is essentially similar. Below the lower transverse margin of the wound, there is depletion of the cambial hormone as a result of its basipetal transport, similar to that obtaining below a complete ring. However, there is still a supply of this hormone arriving from above by way of the longitudinal bridge, below which it spreads out obliquely basipetally on both sides, from the region of relatively high concentration in the



same longitudinal line as the bridge, into the depleted region below the lower transverse margin of the wound. The question then arises, why is there no cambial activity in the region A (text - figure 2, pg. 12), whereas cambial activity occurs lateral to it, where presumably, the concentration of cambial hormone must be lower? In order to explain this, the writer suggests that the wound substance, emanating from the lower transverse wound margin, is used up when it reaches and interacts with the cambial hormone, at what might for convenience be termed the "diffusion" front of the cambial hormone, in terms of which, the absence of cambial activity in region A is again to be interpreted as the result of lack or low concentration of wound substance acting as a limiting factor. At points lateral to A, where cambial activity does occur, but which are equally far removed from the lower transverse margin of the wound as A, wound substance has not been limiting, simply because it has been free to move down that far before meeting and interacting with the cambial hormone at its obliquely basipetal "diffusion" front.

The exceedingly interesting behaviour observed in the modified zig-zag bridge (text - figure 9, pg. 27) can also be readily interpreted along the same lines, in the following manner. The obliquely basipetal development of cambial activity across the lower vertical strip is the result of interaction between the wound substance emanating from the transverse margin just above it, and the cambial hormone at its obliquely basipetal "diffusion" front, in exactly the same manner that obtains below the bridge in the same specimen. The basipetal development down the shorter longitudinal margin of the lower vertical strip is similar to that within a longitudinal bridge, and the absence of cambial activity in the region A (text-figure 9,

pg. 27) is again due to a low concentration of wound substance acting as a limiting factor. There would seem to be little doubt in this case, but that the concentration of cambial hormone must be higher in region A than at points lateral to it on the left, where cambial activity does occur. It is suggested that the wound substance emanating from the transverse margin of the lower vertical strip is used up in interaction with the cambial hormone just above, and lateral to the left of region A, and there would seem to be little possibility of wound substance reaching this region from the longitudinal margin to the right, since what little of it is produced there (c.f. longitudinally bridged wound) is used up in the basipetal development of cambial activity down that margin. To summarise then, local cambial activity in relation to bridged wounds and modifications of such, is to be interpreted as the result of interaction between a wound substance, produced subsequent to wounding; and the cambial hormone which is present in the living bark and moves basipetally down the shoot. At certain points in relation to a wound, a low concentration of wound substance may act as a limiting factor, but its limiting effect is only relative, since cambial activity is further promoted at all points by increased supplies of the cambial hormone (c.f. quantitative experiments).

Although interaction between a wound substance and a hormone is, so far as the writer is aware, a new interpretation of local cambial activity in relation to wounds, the idea is not without precedent with regard to certain other growth processes. Haberlandt (6, 8) found that cell division at the cut surface of potato tubers was most marked in the immediate vicinity of vascular strands. The xylem of the bundle did not seem to play any part, all that was

necessary was phloem tissue. Pieces of potato which did not contain any vascular tissue also showed cell division, but not to the same extent as when phloem was included. If a piece without bundles was separated from another piece with bundles, by means of a thin film of agar, then cell division at the surface of the former was increased. He came to the conclusion that cell division, in pieces of tissue containing vascular bundles, was the result of interaction between a hormone produced in the phloem and a wound hormone produced subsequent to wounding. A summary of Haberlandt's work along these lines is also to be found in a paper by Pringsheim (17). Then again, Nakano (13) came to the conclusion that besides correlative influences, the interaction of two hormones was also involved in the production of typical callus tissue. On the other hand, it is of interest to find that Cholodny (3) has attempted to explain certain experimental results by postulating that a wound hormone retarded growth, by neutralisation of the effect of the growth hormone.

Haberlandt (7, 8) found that he was able, not in every case however, to limit the amount of cell division subsequent to wounding, by washing the wound surface thoroughly in a stream of water. The present writer performed similar experiments, which need not be described in detail here, with wounded balsam poplar. The wound surface was washed from ten to fifteen minutes in a stream of tap water, and then for a few minutes in distilled water. No evidence was obtained that the amount of cambial activity in relation to the wound was in any way affected as a result of washing, and the extent of dying subsequent to wounding was, as far as could be determined, of

the same order in washed and unwashed wounds.

In this present paper the writer has refrained from designating the wound substance as a wound hormone, since apparently, there is some doubt as to the legitimacy of so doing. Petri (14), in a recent paper, concluded that the mode of origin, and properties of so called wound hormones seemed to preclude of their being so defined, even according to the concept of plant hormones. He has suggested that they be considered as an oxidation product of a compound normally present in living cells.

Before concluding, a few remarks relating to further research on the subject of local cambial activity as a result of wounding might be in order. At the present time much is becoming known about the isolation and purification of plant hormones, and in some cases synthesis has been effected. Snow (23) has succeeded in promoting cambial activity by the use of pure hormones. The response was obtained just below the point of application of the hormone. With regard to wound cambial activity on the other hand, the ideal experiment would be one in which a response is obtained in the immediate vicinity of the wound, as a result of application of the hormone at a point some distance from the wound, and in which the response at the wound is more or less distinct and separate from any development arising at the point of application. Stimulation of root development in regions considerably removed from the point of application of the growth hormone has been obtained by a number of investigators, some of whom have already been mentioned (c.f.

Laibach, Thimann and Koepfli, and Cooper), but nothing is known about the response of the cambium in these experiments. Should however an experiment of the type just outlined be successfully performed, the whole problem of certain other relationships, discussed in this paper in terms of interaction between the cambial hormone and a wound substance, would still remain, so that further experimentation is called for in this connection also, in an attempt to determine more definitely the nature of the wound stimulus. The writer himself hopes to be able to continue his experiments along the lines indicated, and it may be that the results obtained in this investigation, and the interpretations suggested are of sufficient interest to stimulate research by other workers.

Summary

- (1) The greater the amount of living bark distal to a wound, the greater is the development of local cambial activity in relation to the wound, in leader shoots of the balsam poplar.
- (2) Local wound cambial activity is promoted by the presence of developing buds and leaves distal to the wound, and the effect can be observed before the basipetal gradient of normal cambial activity emanating from the developing extension growth has reached the wound.
- (3) Cambial activity in relation to wounding responds to gravity in the same way as normal cambial activity. In horizontally placed leader shoots, cambial activity is greater in relation to a wound on the upper side of the shoot than to a similar wound directly opposite on the lower side.
- (4) It is suggested that these results indicate that a hormone pre-

sent in the living bark, and produced by developing buds and leaves, is involved in local cambial activity in relation to wounding. In all probability, this hormone is identical with that which promotes normal cambial activity.

(5) It is postulated that a wound substance, capable of promoting by itself cell division only in the cambial layers, is also involved in local wound cambial activity. The amount of this wound substance produced is proportional to the extent of dying of the cells of the bark subsequent to wounding.

(6) From the lower edge of a complete ring, a very feeble basipetal gradient of cambial activity arises, in which differentiation to form vessels and fibres does not occur, although a few tracheids may be found. This type of behaviour has not been reported hitherto. It is interpreted as the result of stimulation of the cambial layers by the wound substance alone.

(7) Cambial activity in relation to bridged wounds and modifications thereof, involves differentiation of more or less typical vessels and fibres, and is interpreted as the result of interaction between the wound substance and the cambial hormone travelling basipetally in the living cells of the bark.

(8) The absence or feeble development of cambial activity, at certain points in relation to bridged wounds, in contrast to greater development at other points where presumably, the concentration of cambial hormone must be less, is interpreted as the result of lack or low concentration of wound substance acting as a limiting factor.

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Plate 1

- Figure 1. Three units of balsam poplar in three-year-old wood obtained from the same leader shoot, illustrating the relationship, in terms of xylem formation, between the extent of cambial activity in the vicinity of a wound and the amount of living bark distal to the wound. Nat. size.
- Figure 2. Illustrating in longitudinal and transverse section, the effect of gravity upon local cambial activity just distal to a complete ring, as revealed when the bark is peeled from the shoot. Cambial activity is more marked on the upper side. X 2.

Plate 2

- Figure 1. Illustrating wound cambial activity just below a complete ring. Transverse section of a peeled shoot just below a complete ring, showing several layers of more or less regularly rectangular cells cut off to the inside by the vascular cambium. X 160.
- Figure 2. A sheet of xylem from the obliquely basipetal development below a bridged wound. X 110.
- Figures 3 & 4. Xylem formation at corresponding points within the bridge of a longitudinally bridged ring in two contrasting units. In one unit (Figure 3) two buds at the distal end were allowed to develop, whereas the other unit (Figure 4) was completely disbudded. About a third of the bridge, cut transversely, is shown in each photograph. X 120

PLATE 1

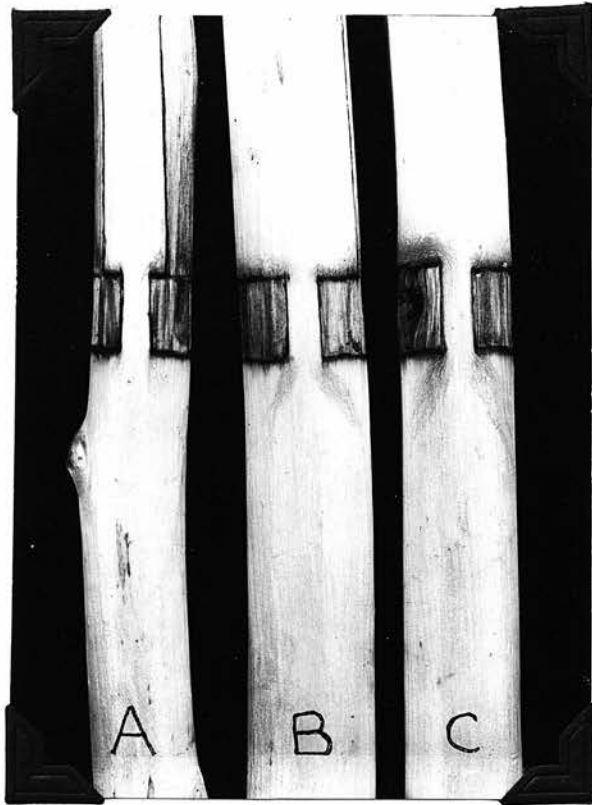


Figure 1

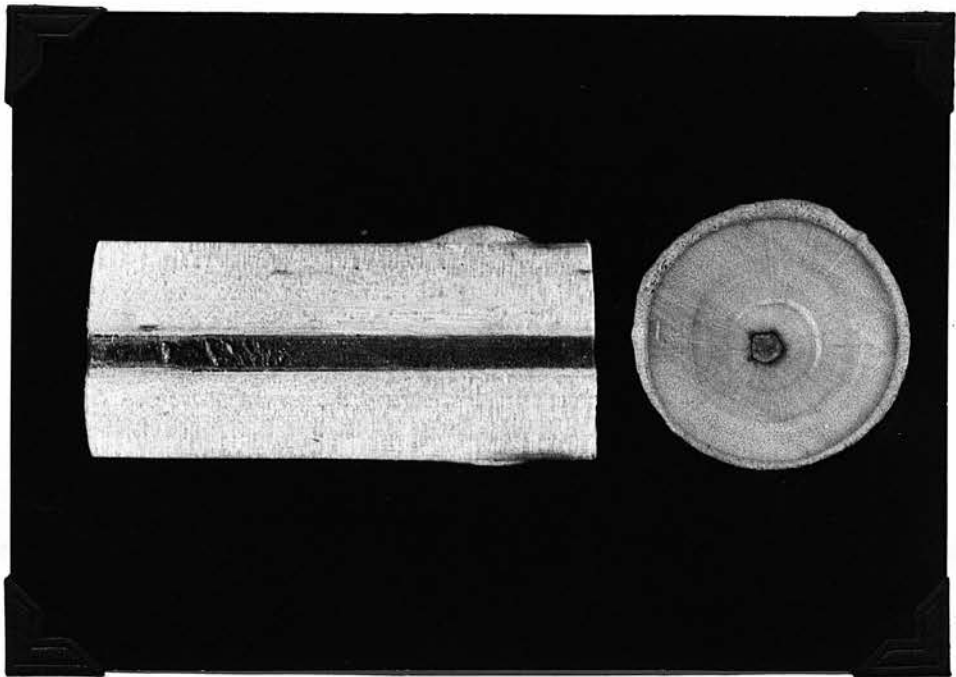


Figure 2

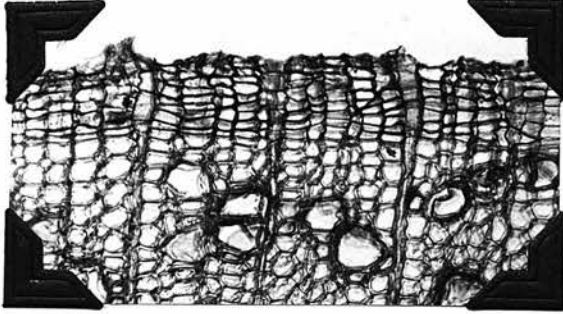


Figure 1

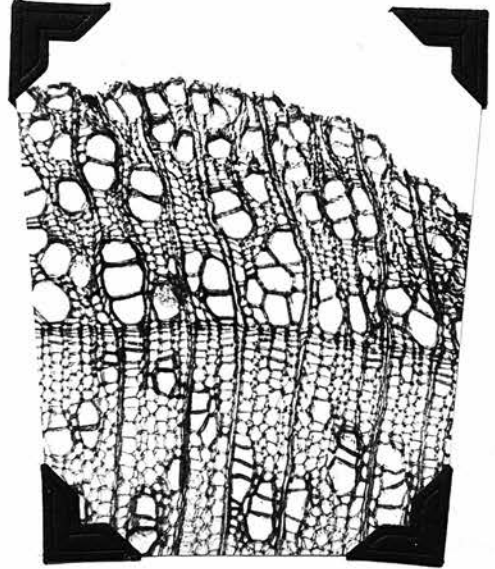


Figure 3



Figure 2

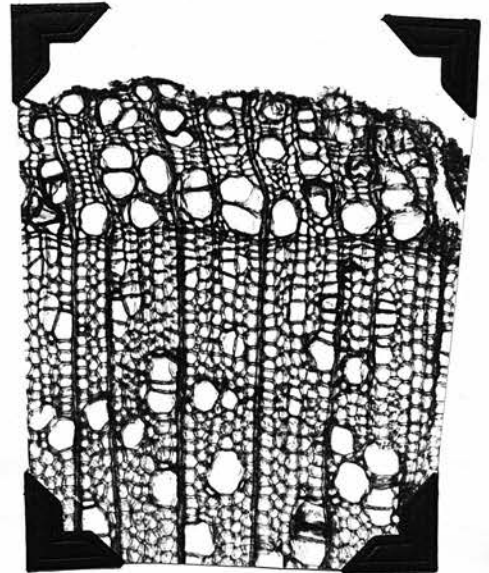


Figure 4

OSMOTIC RELATIONS OF PLANT CELLS, WITH PARTICULAR REFERENCE TO THE
EFFECT OF INTERCELLULAR PRESSURE ON THE GUARD CELLS OF STOMATA.

A subsidiary paper submitted, in addition to a major thesis,
to the University of Edinburgh for the degree of Doctor of Science.

BY

ALEXANDER BINNIE BROWN, B. SC. (EDIN.), M. SC. (M^CGILL)

Lecturer in Botany at the University of Alberta, Edmonton, Canada.

1936

Note: This paper was submitted to the editor of the *Annals of Botany* who expressed a willingness to publish, only if the theoretical portion were very much reduced. This the author does not feel inclined to do, since he feels that he has a definite contribution to make towards a clearer understanding of the osmotic relations of plant cells. Negotiations are now under way to have the paper published as it is here presented.



OSMOTIC RELATIONS OF PLANT CELLS, WITH PARTICULAR REFERENCE TO THE
EFFECT OF INTERCELLULAR PRESSURE ON THE GUARD CELLS OF STOMATA

BY

A. B. BROWN

INTRODUCTION

This research was suggested by the recent work of Ernest (2), who demonstrated experimentally the effect of intercellular pressure on the suction pressure of plant cells. She showed that when mesophyll cells were removed from a fully turgid leaf and immersed in water, further absorption of water took place. This suction pressure was estimated in the usual way, by determining the molar concentration of a sucrose solution in which the cells neither increased nor decreased in volume. The corresponding value in atmospheres, she interpreted as being a measure of the maximum intercellular pressure, defined as the mutual pressure of surrounding cells, which resists full expansion of individual cells in their normal situation in the leaf. As a result of this work, she has suggested that the suction pressure equation be amended to take the form, $S_p = O_p - (W_p + A)$, where S_p is the suction pressure, O_p the osmotic pressure, W_p the wall pressure, and A the sum of the intercellular pressures.

Still more recently, Ursprung (6) has also modified his suction pressure equation, as applied to plant cells subjected to foreign mechanical strain or stress, to read as follows, $S_p = O_p - (W_p \pm A)$, ($+A$) if an external pressure, and ($-A$) if a tension is present. He gives no further details, neither does he indicate that values for ($+A$) or ($-A$) have been determined experimentally.

In this present paper experiments will be described, which demonstrate clearly the effect of intercellular pressure upon the guard cells of stomata, under certain conditions. Before proceeding to a description of the experimental work however, it is proposed to analyse critically some of the concepts involved in the above equations, because it is felt that this is still a real necessity, and also in order to provide a background for discussion of the experimental results.

EXAMINATION OF CONCEPTS

(1) Suction pressure of the cell (S_p)

Ursprung has defined the suction pressure of a cell (S_p), as the force per unit area with which the whole cell, consisting of the cell wall and the contents of the cell, tends to absorb water (6. pg. 116). Again (6. pg. 122 and 7. pg. 202), he particularly stresses the point that the suction pressure of a cell equals the force with which the cell strives to take in water. With respect to the value of this force, the writer is in agreement with Ursprung, but his definition of it is open to criticism. Consider an isolated cell immersed in water. At equilibrium, the osmotic pressure of the cell contents is exactly balanced by the wall pressure, the cell cannot absorb any more water, and its suction pressure (S_p) is zero; $O_p - W_p = 0 = S_p$. The ability of the cell to absorb more water, under the prevailing conditions, is zero, but surely the tendency for water to enter the cell is equal to O_p , the osmotic pressure of the contents of the cell. If the cell wall is stretched, the cell will lose water when it is immersed in a harmless non-penetrating solution. Once the cell is again in equilibrium with an external solution, the tendency for water to enter the cell, from the external solution, is equal to the difference between the osmotic pressure of the cell contents (O_p), and the osmotic pressure of the external solution. This is equivalent to the turgor pressure (T) of the cell. The equivalent in atmospheres of the molar concentration of the external solution is a measure of the

suction pressure of the cell (S_p), relative to pure water. If the cell is now transferred from the external solution to pure water, water will not simply tend to enter, but actually will enter, and the suction pressure of the cell (S_p) is a measure of the force per unit area with which water begins to enter the cell. As water is absorbed, the osmotic pressure of the cell contents (O_p) decreases, the wall pressure (W_p) increases, and the force per unit area with which water enters (S_p) continually decreases, until the tendency for water to enter is exactly balanced by the wall pressure. At this point equilibrium is attained, and

$$O_p - W_p = 0 = S_p.$$

The tendency for water to enter a fully turgid cell would be clearly expressed as equal to the osmotic pressure of the cell contents (O_p), if a pressure gauge or manometer were attached to the cell, and in contact with the cell contents. Now one might reply that this is a measure of the tendency for water to enter the cell contents only, whereas the tendency for water to enter the whole cell including the cell wall is zero. This is really the point at issue. When the osmotic pressure of a solution in an osmometer is balanced by a column of mercury, one conceives of the mercury column as balancing exactly the tendency for water to enter the osmometer, and it is of course a measure of the osmotic pressure of the solution within the osmometer. In such a case one does not, and cannot, postulate that the tendency for water to enter the solution within the osmometer is equal to the osmotic pres-

sure of that solution, whereas the tendency for water to enter the osmometer and the included solution, considered as one unit, is zero. However, this seems to be what Ursprung and most others are doing with respect to the plant cell. Such a distinction cannot be made. If the tendency for water to enter a fully turgid cell (or a balanced osmometer) were zero, then the tendency for water to enter the contents of the cell would also be zero, under the prevailing conditions. On the other hand, if the tendency for water to enter the contents of a fully turgid cell is equal to the osmotic pressure of the cell contents, then the tendency for water to enter the cell as a whole must also be equal to the osmotic pressure of the cell contents. This latter is the true state of affairs. If the cell wall were of unlimited extensibility and offered no resistance to entrance of water into the cell, the tendency for water to enter the cell would always be equal to the osmotic pressure of the cell contents at any moment. However, if the cell wall is of limited extensibility and does offer resistance to absorption of water, there will come a time when the tendency for the cell (cell wall + protoplasm + cell sap) to absorb water is exactly balanced by the resistance offered by the cell wall. Nevertheless at that point, a tendency for water to enter the cell still exists, and the mere fact that it is exactly balanced by the wall pressure does not automatically dissolve that tendency, which is equal to the full osmotic pressure of the cell contents. The ability, however, either of a fully turgid cell, or of the contents of such a cell to absorb

more water is zero, under the prevailing conditions. The tendency for water to enter a fully turgid cell, in equilibrium with pure water externally, would be zero only if the cytoplasm became impermeable to water.

It is therefore suggested that the suction pressure of a cell (S_p) should be defined, not as the force per unit area with which the cell tends to absorb water, but as the force per unit area with which water begins to enter the cell, when the cell is immersed in pure free water. It is necessary to specify "free" or "completely available" water in the above definition, because if the external water were under tension, (cf. Ursprung 6. pg. 118, fig. C), a totally different state of affairs would exist. Moreover, in the case of a cell which has lost water to the atmosphere, the immediate external environment may be pure water, but the water is held in the cell wall by imbibitional forces, and is not completely available (cf. Thoday 5, pg. 122). In the above definition, "begins" in itself implies that the force is a constantly changing one as water is being absorbed, and "when the cell is immersed in pure free water" in itself implies that the force may be potential.

(2) Osmotic pressure of the cell contents (O_p)

At any moment, the osmotic pressure of the cell contents (O_p) is the equivalent in atmospheres of the molar concentration of a sucrose solution, isotonic with the cell contents. No difficulty need be experienced with this concept, if it is remembered that the force is always relative to pure free water. It can be defined as the force per

unit area with which water would initially tend to enter an unsaturated cell, when such a cell is immersed in pure free water. If the cell is saturated and in equilibrium with pure free water, O_p is a measure of the tendency for water to enter the cell, and is exactly balanced by the wall pressure.

(3) Turgor Pressure (T)

Turgor pressure is the actual pressure exerted upon the cell wall by the cell contents at any moment, and is, according to the present writer, a measure of the force per unit area with which water from the external environment tends to enter the cell at any moment. It is numerically equal to the osmotic pressure of the cell contents (O_p), only when the cell is bathed by pure free water. At any equilibrium, the turgor pressure is equal to the wall pressure (W_p) which acts in the opposite direction. It may however be resolved into two components in the following manner. Let us consider a specific example cited by Ursprung (6). $S_p = O_p - W_p = 9.7 - 5.4 = 4.3$ atm. This is the relationship obtaining for a pith cell of Impatiens, either in its normal condition in situ within the plant at a particular moment, or isolated and at equilibrium in liquid paraffin, or immersed in a sucrose solution with an osmotic pressure of 4.3 atmospheres, in which it retains its original volume. Equilibrium may be represented as $O_p - S_p = W_p$, or simply as $T = W_p$, where T is the turgor pressure. $T = O_p - S_p$ numerically. O_p is a measure of the osmotic pressure of the cell contents relative to pure water; S_p , the suction pressure of the cell relative to pure water, may be conceived of as being a

measure of the extent to which the external water is not pure or free, and T is a measure of the tendency for water to enter the cell, under the conditions prevailing at the equilibrium under consideration.

When the cell is in situ within the plant, or immersed in liquid paraffin, S_p (4.3 atm.) is a measure of the force per unit area with which the water external to the protoplast is held within the cell wall.

(4) Wall pressure (W_p).

This is usually conceived of as being the pressure which the stretched wall exerts upon the cell contents. At any equilibrium it is numerically equal to the turgor pressure (T). Now Ernest (1) has shown that, in some xerophytic and aquatic plants, the cell wall is rigid and does not stretch. In such a case, wall pressure is not of the nature of an active inwardly directed pressure tending to squeeze water out, but rather a static resistance preventing absorption of water by the cell. The volume of such a cell is independent of the osmotic pressure of the cell contents (O_p). The suction pressure of such a cell is always zero, unless the contents are withdrawn from the cell wall, when the virtually naked protoplast has a suction pressure equal to its full osmotic pressure. On the other hand, such a cell will not lose water to an external solution, until the osmotic pressure of the external solution becomes greater than the osmotic pressure (O_p) of the contents of the cell.

In the typical plant cell with an extensible cell wall, in situ within a tissue, there are other theoretical possibilities over and above the situation where the cell wall is exerting an active inward-

ly directed pressure on the cell contents. For example, the cell wall may not be stretched at all, if the turgor pressure within a cell is exactly balanced by the mutual pressure of surrounding cells, which resists expansion of the cell under consideration. Moreover, a cell may be compressed by surrounding cells, and although the wall of the compressed cell is stretched, it is by the turgor pressure of the surrounding cells, and it will exert an outwardly directed pressure tending to cause water to enter the compressed cell. This possibility is discussed in the next section on intercellular pressure. Still another possibility exists. If a cell wall is extensible, it may also be compressible, and we have the possibility of a cell compressed by surrounding cells in such a way, that the cell wall of the compressed cell is itself compressed. Here again the result would be, that the compressed cell wall exerts an outwardly directed pressure, tending to cause water to enter the compressed cell. This, according to the present writer, is the situation to be found in the walls of guard cells of stomata, under certain conditions.

(5) Intercellular pressure.

An attempt will be made to analyse this concept by means of simple theoretical models. Text - figure 1 represents two artificial cells with the following characteristics. All the walls are rigid and inextensible, except the two walls AB and CD which are in contact and are extensible. All the cell walls are freely permeable to water and to substances in solution. One can imagine that, just as in the normal plant cell, there is a lining layer of cytoplasm, the seat of

semipermeability, and a central vacuole. The model is immersed in pure water, and at equilibrium the osmotic pressure of the contents of cell X is 18 atmospheres, and of cell Y 20 atmospheres. The walls AB and CD are stretched, and their position is determined by the pressure difference of 2 atmospheres in the two cells.

If cell X were killed, cell Y would absorb water, and the walls AB and CD would be distended further, until a new equilibrium was reached. In other words, cell X when alive exerts intercellular pressure upon cell Y. This could be measured experimentally by determining the concentration of an external solution, in which Y would neither increase nor decrease in volume after the death of X, and obviously it would require an external solution with an osmotic pressure equal to 18 atmospheres. The equilibrium in water, for cell Y, when both cells are alive could be represented as follows:

$$O_p - (W_p + A) = 20 - (2 + 18) = 0 = S_p.$$

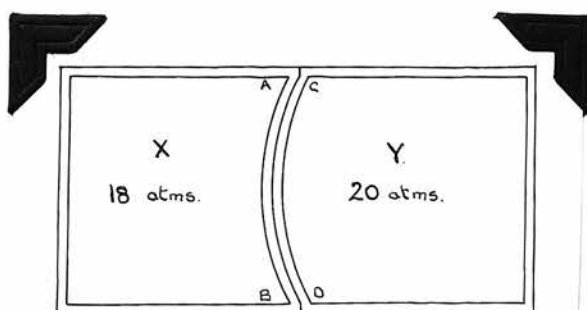
Now, if instead of simply killing cell X, imagine that it is completely removed. What would now be the osmotic pressure of the external solution, necessary to maintain cell Y at its original volume? Obviously it would require a solution with a greater osmotic pressure than 18 atmospheres, because the wall AB has been removed, and a pressure difference of two atmospheres would cause further stretching of the single wall CD. This means that one has to distinguish between the intercellular pressure of the contents of X, and the intercellular pressure of the complete cell. Moreover, if after cell X is killed and the new equilibrium in water attained, the dead cell X is then re-

moved, further absorption of water by Y would again take place. In other words, the wall AB itself exerts "intercellular" pressure on cell Y. A pertinent question that arises in this connection is, how should one represent the equilibrium between, for example, an isolated endodermal cell or an epidermal cell and water, $Op - Wp = 0 = Sp$, or $Op - (Wp + A) = 0 = Sp$, where A is the resistance to full expansion offered by the Casparian strip in the one case, and the cuticle on one of the walls in the other?

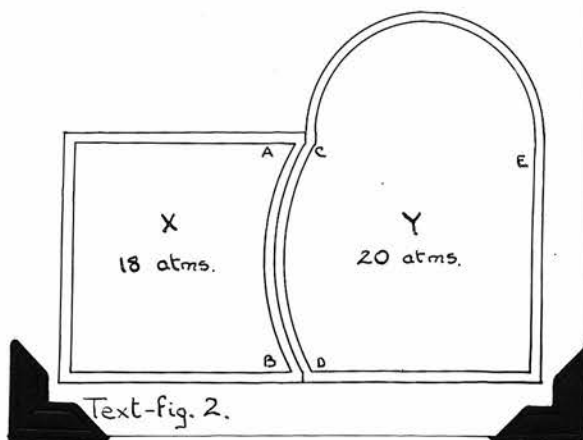
Consider now what would happen if cell Y were killed. It would be necessary, theoretically, to immerse the model in a solution with an osmotic pressure of 20 atmospheres to maintain the volume of cell X unchanged, and 20 atmospheres would be a measure of the intercellular pressure of the contents of Y exerted upon cell X. The relationship obtaining for cell X, when both cells are alive and in equilibrium with pure water, could be represented as follows:

$$Op - (-Wp + A) = Op - (A - Wp) = 18 - (20 - 2) = 0 = Sp.$$

However, if X and Y were typical plant cells and cell Y were killed, and if under those conditions, the model were immersed in an external solution with an osmotic pressure of 20 atmospheres, a totally different result would be obtained. The tension in the walls AB and CD would be released, they would contract, and the protoplast of cell X would lose water to the external solution of greater osmotic pressure. The protoplast of cell X would lose water to any non-penetrating external solution with a greater osmotic pressure than 18 atmospheres. Nevertheless, the relationship between the two living cells X and Y



Text-fig. 1.



Text-fig. 2.

Text - figures 1 and 2. Explanation in text.

and pure water externally, can still be represented as above. Notice that a general equation applicable to both cells could be written as follows, $S_p = O_p - (A \pm W_p) = 0$, ($+ W_p$) where wall pressure is positive, tending to squeeze water out, and ($- W_p$) where wall pressure is negative, tending to pull water into the cell. Now Ursprung's general equation (6) reads, $S_p = O_p - (W_p \pm A)$. This implies a positive wall pressure tending to squeeze water out of the cell, and an external force tending to cause water to enter the cell. In the opinion of the present writer, such a relationship is inconceivable.

Text - figure 2 depicts a model essentially similar to that already discussed, with the exception that the wall CE is extensible as well as AB and CD. All the other walls are rigid. The model is in equilibrium with pure water externally. Cell Y may be compared, in a rough way, with a plant cell in contact with a neighbouring cell at one part, and bordering on an intercellular space at another part of its surface area. If cell X is killed or removed, and as a consequence, Y absorbs more water, X must exert intercellular pressure on Y when both cells are alive. Consider now the equilibrium with water as shown in the figure. The tension in the wall CE is counterbalancing exactly the osmotic pressure of the contents or turgor pressure of cell Y, i.e., $W_p = O_p = T$, and $S_p = O_p - W_p = 0$. The pressure per unit area, exerted by the contents of cell Y upon the wall CD, is precisely the same as that exerted upon the wall CE, but in terms of the amended suction pressure equation, $S_p = O_p - (W_p + A) = 0$, or $T = W_p + A$. In other words, wall pressure (W_p) must vary at dif-

ferent points in the same cell. If the wall CE were in contact with another cell Z, the osmotic pressure of whose contents (O_p) differed from that of X, intercellular pressure (A) exerted upon cell Y would also vary at different points in the same cell, at the same moment. It would appear, therefore, to be impossible to ascribe to cell Y, while it is in contact with the living cell X, a wall pressure (W_p), or an intercellular pressure (A) characteristic of the cell as a whole. The turgor pressure (T) would however be characteristic of the cell, and theoretically measurable. Undoubtedly, the same situation prevails in a plant cell in situ within a tissue, particularly if it has different degrees of contact with intercellular spaces and neighbouring cells.

Another point worthy of note in connection with the second model, is that if cell Y absorbs more water on the death or removal of cell X, the turgor pressure of Y must fall, resulting in a decrease in the pressure difference inside and outside the cell. It follows therefore, that the wall CE will contract when this happens, and in terms of the amended suction pressure relationship, we are asked to conceive of the wall pressure (W_p) decreasing in some parts and increasing in other parts, as the cell absorbs water.

In view of the above considerations, it seems clear that any attempt to insert the concept of intercellular pressure (A) into the suction pressure equation involves certain difficulties. On the other hand, it is equally clear that the action of intercellular pressure is of extreme importance. However, further discussion al-

ong these lines is reserved for the present, and experiments will now be described, which show that the action of intercellular pressure may be even more complex than is as yet apparent.

EXPERIMENTATION

Tangential sections were made from the under surface of leaves of various plants, which had been kept in darkness for at least twenty-four hours. The sections were washed in a large volume of distilled water, in which they were left for several hours in the dark, and in some cases over night. Measurements were then made, in distilled water and gradually increasing concentrations of sucrose, of selected stomata falling into two groups; those surrounded by living epidermal cells, and those surrounded by epidermal cells which had died. The instrument used was an eye-piece screw micrometer. Except in the case of distilled water, the cover slip was ringed with melted vaseline to prevent loss of water and consequent change in concentration of the sugar solution, during the time that measurements were being made. It is known of course that stomata are sensitive to light. In these experiments the sections were exposed to light, only during the time that the measurements were being made. About ten stomata were selected, and in any one medium the stoma measured first was again measured after the others. No change was ever found to have taken place during the time that measurements were being made. Indeed tests were made with sections exposed on the stage of the microscope to the source of illumination used, an ordinary electric bulb, and even after as long as four hours no change in stomatal posture was observed. It is therefore assumed that the results obtained are not complicated

by the action of light. Only the widths of the stomata are given, since it was found that in all the material worked with, the length of the stomata remained constant.

In tables 1 and 2 are given results representative of the behaviour of the stomata of Anthericum liliago, on being passed from distilled water through increasing concentrations of sucrose. The figures given are those of stomatal width, and the unit of measurement is equal to 0.8μ . The numbers in brackets indicate the increment or decrement relative to the preceding value. It will be observed that there is a marked difference in stomatal width in water, accordingly as the surrounding epidermal cells are dead or alive. If the epidermal cells are dead, the stomata are definitely open and a considerable stomatal pore (not measured) is visible, and just as one would expect in this group, stomatal width decreases and the pore gets smaller in gradually increasing concentrations of sucrose. Beyond a certain concentration the width becomes constant. This occurs when the wall tension in the guard cells has been reduced to zero, and plasmolysis follows. But if the surrounding epidermal cells are alive, the behaviour of the guard cells in increasing concentrations of sucrose is indeed remarkable. The stomata increase in width in this case. Ultimately a constant width is attained, after the wall tensions in the guard cells have been reduced to zero, and plasmolysis of the guard cells follows. Once plasmolysis has occurred, the widths of the stomata in the two groups are very similar, although there is a tendency for the widths

to be slightly greater where the surrounding epidermal cells are dead. It may be stated at this point that the action is completely reversible. This can be seen very readily, if a strongly plasmolysed section is mounted in water and observed under the microscope. After deplasmolysis of the epidermal cells has taken place, a very marked compression of the guard cells follows, of such a degree as to be easily visible without the aid of any measuring instrument. This applies to stomata surrounded by living epidermal cells only; if the epidermal cells are dead, the amplitude of movement of the stomata is too small to be visible without the aid of the micrometer.

Now in the case of stomata where the surrounding epidermal cells are dead, one associates decrease in width, in increasing concentrations of sucrose, with loss of water by the guard cells. What then of the stomata that increase in width in increasing concentrations of sucrose? Do they absorb water? On the basis of the following considerations, the writer suggests that they do. Stomatal length is constant, variation in depth of the guard cells could not be detected, and there was no evidence forthcoming that simply change of shape and not volume was involved. For the present then, it is assumed that the guard cells surrounded by living epidermal cells absorb water, on being transferred from distilled water to suitable concentrations of sucrose. It is hoped to show later that this is not only possible, but actually to be expected, under the prevailing conditions.

TABLE 1

Anthericum liliago: Width of stomata in water and different molar concentrations of sucrose.

H ₂ O	.05	.10	.15	.20	.25	.30	.40	Remarks
23.7	24.4 (0.7)	26.0 (1.6)	26.6 (0.6)	29.1 (2.5)	32.5 (3.4)	34.5 (2.0)	34.5	Epidermal cells alive.
23.1	24.3 (1.2)	25.3 (1.0)	26.8 (1.5)	28.6 (1.8)	31.2 (2.6)	32.5 (1.3)	32.4	
25.7	26.6 (0.9)	27.1 (0.5)	28.4 (1.3)	30.8 (2.4)	34.3 (3.5)	35.1 (0.8)	35.3	
24.5	25.5 (1.0)	26.4 (0.9)	28.2 (1.8)	29.8 (1.6)	32.7 (2.9)	35.4 (2.7)	35.5	
25.9	26.7 (0.8)	27.6 (0.9)	28.9 (1.3)	30.5 (1.6)	33.1 (2.6)	35.6 (2.5)	35.4	
40.6	39.4 (1.2)	39.2 (0.2)	38.3 (0.9)	37.4 (0.9)	37.3	37.1	37.1	Epidermal cells dead.
43.0	42.3 (0.7)	41.6 (0.7)	40.4 (1.2)	39.5 (0.9)	39.1	39.1	39.1	
39.5	38.6 (0.9)	38.4 (0.2)	37.2 (1.2)	36.8 (0.4)	36.5	36.5	36.3	
40.5	39.7 (0.8)	39.3 (0.4)	38.7 (0.6)	37.7 (1.0)	37.5	37.3	37.2	
42.2	41.5 (0.7)	40.3 (1.2)	38.7 (1.6)	38.4 (0.3)	38.4	38.3	38.5	

TABLE 2

Anthericum liliago: Width of stomata in water and different molar concentrations of sucrose.

H ₂ O	.19	.21	.23	.25	.27	.29	.30	.40	Remarks
24.8	27.9 (3.1)	28.6 (0.7)	29.4 (0.8)	30.1 (0.7)	32.8 (2.7)	35.2 (2.4)	35.9 (0.7)	35.8	Epidermal cells alive.
25.3	27.8 (2.5)	28.6 (0.8)	29.2 (0.6)	30.4 (1.2)	32.7 (2.3)	34.9 (2.2)	36.0 (1.1)	36.0	
24.2	27.9 (3.7)	28.5 (0.6)	29.4 (0.9)	30.4 (1.0)	32.2 (1.8)	35.0 (2.8)	35.8 (0.8)	36.0	
25.3	28.3 (3.0)	29.0 (0.7)	30.1 (1.1)	31.9 (1.8)	33.9 (2.0)	35.6 (1.7)	35.8 (0.2)	35.8	
24.0	27.8 (3.8)	28.4 (0.6)	29.5 (1.1)	31.1 (1.6)	33.7 (2.6)	34.5 (0.8)	35.0 (0.5)	35.2	
41.6	38.6 (3.0)	37.7 (0.9)	37.7	37.6	37.7	-	-	37.7	Epidermal cells dead.
41.6	38.3 (3.3)	38.0 (0.3)	38.1	38.2	38.2	-	-	38.1	
40.7	38.0 (2.7)	36.8 (1.2)	36.6	36.5	36.4	-	-	36.4	
40.8	37.2 (3.6)	36.3 (0.9)	36.5	36.4	36.4	-	-	36.4	
42.1	38.3 (3.8)	38.1 (0.2)	38.0	38.0	38.1	-	-	38.1	

Observations on the incidence of visible plasmolysis were also made in these experiments. When the epidermal cells are dead, plasmolysis occurs in the guard cells long before it does in guard cells surrounded by living epidermal cells. In the case of guard cells surrounded by dead epidermal cells, plasmolysis can be observed in .25M sucrose, but it is by no means obvious. Notice in this connection (Table 2) that the widths of the stomata in this group are constant from .21M sucrose onwards. This indicates that the wall tension is reduced to zero some considerable time before incipient plasmolysis can be observed. One would rather expect this in cells such as guard cells, which are small, and in which the protoplasmic content is high, relative to the volume of the cell. If the epidermal cells are alive, plasmolysis of the guard cells and the epidermal cells becomes definitely visible in .30M sucrose. In .29M sucrose no plasmolysis is visible, nor is there any evidence that the wall tensions are reduced to zero at this concentration, since there is still a marked change in stomatal posture on being transferred from .29M to .30M sucrose. In other words, there is not the same lag between attainment of constant width and the incidence of incipient plasmolysis when the surrounding epidermal cells are alive, as when the epidermal cells are dead. This is not surprising if, as has been suggested, the volume of the guard cell increases with increase in concentration of the external sucrose solution.

The increase in width for equal increments in molar concentration of sucrose is of interest. To begin with, the increase in width is small, it increases ultimately to a maximum and then decreases. Most of the movement takes place in the higher concentrations of sucrose. On a priori grounds, which need not be discussed here, this is more or less what one would expect.

One other point is worthy of mention. One occasionally finds guard cells which have died, still surrounded by living epidermal cells. In such a case, as a result of the hydrostatic pressure in the epidermal cells, the wall of the dead guard cell is closely adpressed against its more rigid contents, such as the nucleus and chloroplasts, so that in outline the wall becomes quite anfractuous. This is of interest as being indicative of the high degree of pliability, and the readiness with which the guard cell wall responds to pressure differences.

Similar experiments were performed with Zebrina pendula. Actually the work was first begun with this plant, Anthericum is the result of a search for further suitable material. Tables 3 and 4 are representative of the results obtained. The amplitude of movement is here very much smaller than in Anthericum, and because of the relatively high experimental error, estimated at ± 0.2 , the writer hesitates to analyse the figures to any great degree. Zebrina also differs from Anthericum in that the guard cells are bounded by four well defined auxiliary cells. The stomata are again considered in two groups, those surrounded by living auxiliary cells, and those enclosed by

auxiliary cells which have died. The unit of measurement is the same as before. In distilled water, stomata whose auxiliary cells are dead, are definitely open, a distinct pore is visible, and they decrease in width with increasing concentration of the external sucrose solution until a constant width is attained. If the auxiliary cells are alive, the width of the stomata increases on being transferred from water to sucrose solutions. The point of particular interest (Table 3) is that there are definite indications that in a gradually increasing sucrose solution, the stomata may increase in width up to a maximum and then decrease, before becoming constant. Now experiments have been carried out where this increase in width followed by a decrease is not obvious (Table 4), but on the whole this happened less often.

TABLE 3

Zebrina pendula: Width of stomata in water and different molar concentrations of sucrose.

H ₂ O	.05	.10	.11	.12	.13	.50	Remarks
39.2	40.3	42.5	42.5	42.2	42.3	41.7	Auxiliary cells alive.
38.0	40.3	43.1	41.8	40.5	40.5	40.6	
39.2	41.0	43.2	43.2	42.5	42.6	42.5	
38.8	39.9	42.5	42.0	41.8	41.7	41.5	
38.4	39.3	42.2	41.9	41.7	41.5	41.6	
43.7	42.5	42.4	42.5	42.5	42.3	42.2	Auxiliary cells dead.
44.1	43.0	42.8	42.7	42.8	42.8	42.6	
41.8	41.3	41.3	40.9	40.5	40.6	40.4	
45.5	44.2	44.5	44.1	44.3	44.5	44.2	
47.3	46.7	46.2	46.4	46.5	46.5	46.4	

TABLE 4

Zebrina pendula: Width of stomata in water and different molar concentrations of sucrose.

H ₂ O	.05	.10	.11	.12	.13	.14	Remarks
38.5	39.0	41.7	42.0	42.0	42.8	42.6	Auxiliary cells alive.
39.3	40.0	42.1	43.0	43.0	44.1	43.8	
38.9	40.0	42.4	42.9	43.0	44.0	43.7	
38.3	39.0	41.5	41.6	41.8	42.2	42.1	
38.0	38.8	40.4	41.0	41.1	41.2	39.6	

Observations were again made on the incidence of incipient plasmolysis. Zebrina pendula has already been studied from this point of view by Wiggans (7). He found, when the stomata were shut, that there was very little difference between the molar concentrations of CaCl₂ isotonic with the guard cells and epidermal cells at incipient plasmolysis. His results give .09M CaCl₂ as isotonic with the epidermal cells, and .10M CaCl₂ as isotonic with the guard cells. In one experiment he found no difference at all. With these results, as far as they go, the writer is in agreement. Incipient plasmolysis of the epidermal cells was observed around .20M sucrose, and of the guard cells at slightly higher concentrations on the whole. But with regard to the auxiliary cells, which surprisingly enough, Wiggans does not mention at all, the state of affairs is entirely different. At .20M sucrose the auxiliary cells are markedly plasmolysed, plasmolysis is still evident at .15M sucrose, and has been detected as low as .13M sucrose. The amplitude of movement of stomata with

dead auxiliary cells is so small in Zebrina as to make an estimation of the concentration of sucrose, at which constant width is attained, extremely difficult. But when the auxiliary cells are alive the amplitude of movement is greater, just as in Anthericum, and the evidence here is that constant width is attained in concentrations close to .13M sucrose. Here again we have evidence that an estimation of the concentration of a solute causing incipient plasmolysis may be very definitely greater than that necessary to reduce the wall tension to zero.

Increase in width of stomata surrounded by living epidermal cells, when sections are transferred from water to sucrose solutions has also been observed in other plants, e.g., Geranium, Cyclamen, Begonia, and some others. But the amplitude of movement is small, in Geranium, about the same as in Zebrina, and in the others examined still less. Moreover the material is not nearly so convenient to work with as either Anthericum or Zebrina. However, it is of considerable interest to know that compression of the guard cells, as a result of the intercellular pressure exerted upon them by the immediately adjacent epidermal cells, is not uncommon. Compression here implies that the volume of the guard cells is less than that which obtains when the guard cell walls are at equilibrium, i.e., neither stretched nor compressed. It would not necessarily involve corrugation of the guard cell wall. All that need happen is that the wall become thicker.

The behaviour just described was observed, of course, under abnormal conditions, but there is no doubt, at all events in Anthericum, that compression of the guard cells is a normal feature,

as can be observed simply by examining under the microscope an entire leaf from a plant kept in darkness until the stomata have closed. Scarth (3), in his work on the effect of H-ion concentration on stomata, reports that in Zebrina complete closure did not take place at any pH, if the auxiliary cells were disrupted. In all the plants examined by the writer, a slight slit between some of the guard cells could usually be detected in pieces of plasmolysed epidermis, even if the surrounding auxiliary cells were alive. It is not improbable that the action of intercellular pressure is necessary for complete closure of stomata of the type described above, in which case, the stomata do not simply shut of themselves alone, but are squeezed shut by the action of the immediately adjacent cells.

INTERPRETATION OF RESULTS

That guard cells surrounded by dead epidermal cells lose water on being transferred from water to solutions of sucrose, is just what one would expect, and the mechanism is clear. The writer has already suggested that guard cells surrounded by living epidermal cells absorb water, when a piece of epidermis is transferred from water to suitable concentrations of sucrose. In this case the mechanism is not so evident, and so calls for interpretation. It might be worth while at this point to refer once again to the model depicted in text - figure 1. If such a model were transferred from water to a sucrose solution with an osmotic pressure of, let us say, two atmospheres, the cells would not lose water, the pressure difference of two atmospheres between X and Y would still be maintained,

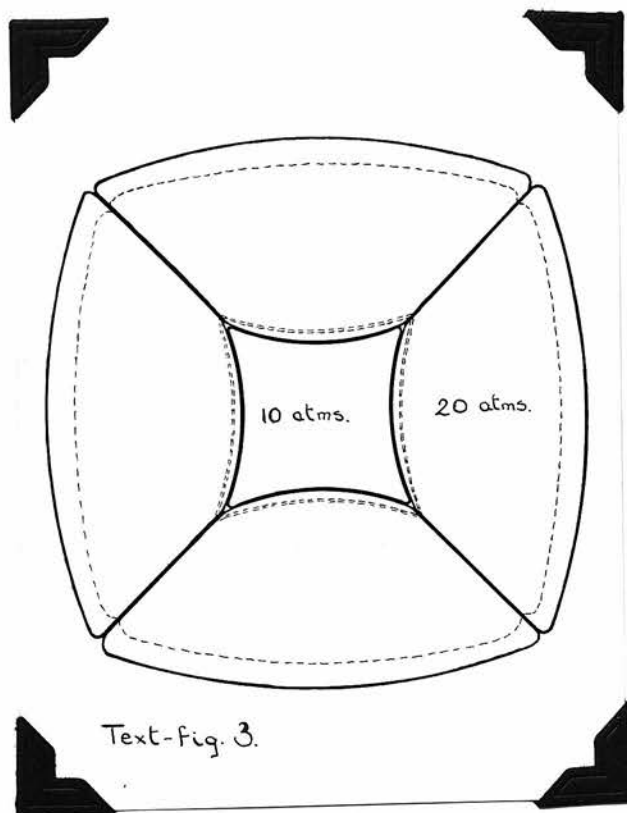
and the position of the walls AB and CD would remain unaltered. It is clear however, that the turgor pressure in both cells would be automatically reduced, to sixteen atmospheres in X and to eighteen atmospheres in cell Y. Undoubtedly, in a piece of epidermal tissue also, the turgor pressure of the cells is automatically reduced on transference from water to a sugar solution, but apparently something else must happen to explain absorption of water by guard cells surrounded by living epidermal cells.

Consider now the system depicted in text - figure 3, which for simplicity will be discussed in terms of two dimensions only. It represents a central cell, the osmotic pressure of whose contents is ten atmospheres, surrounded by four other cells with an osmotic pressure of twenty atmospheres each. The system is in equilibrium with pure water externally. All the cell walls are freely permeable to water and solutes, and again one can imagine that there is a lining layer of cytoplasm, the seat of semipermeability, within each cell wall and surrounding the central vacuole. The cell walls are all stretched, but the central cell is compressed by the surrounding cells. Let the model be now transferred from water to a sugar solution with an osmotic pressure of two atmospheres, assume that the sugar solution comes into contact with all the cells simultaneously, and consider what happens. There appears to be only one possibility. The surrounding cells will lose water, the central cell will absorb water, and a new equilibrium will ultimately be attained as indicated by the broken line in text - figure 3. Now

the difference in turgor pressure between the central cell and the surrounding cells, at this new equilibrium, will be even greater than it was when the system was in equilibrium with water. When the system is transferred from water to the sugar solution, the turgor pressure in the surrounding cells is automatically reduced to eighteen atmospheres, and in the central cell to eight atmospheres, but, as the surrounding cells lose water the concentration of solutes within them increases, whereas in the central cell there is a decrease due to absorption of water. This does not mean that the position of cell walls is not determined by pressure differences between adjacent protoplasts, for at any equilibrium pressure differences between protoplasts undoubtedly do determine the position of extensible cell walls between them. But it does show clearly that a difference in pressure is not the only factor under conditions where water exchange is taking place. The significant point emerging from the above considerations is simply this, that when the surrounding cells lose water, the position in space at which they resist expansion of the central cell changes, or in other words, there is what may be termed a withdrawal of the intercellular pressure exerted upon the central cell by the surrounding cells. Notice that actual separation of the cells, one from the other, is not involved. In the same way, if the model depicted in text-figure 2 were transferred from water to a sugar solution with an osmotic pressure of two atmospheres, cell Y would lose water and cell X would absorb water. Here, withdrawal in space of the resistance to expansion of

cell X, offered by cell Y, is perhaps not so obvious, and indeed it would be very easy, by a fallacious process of reasoning, to arrive at exactly the opposite conclusion with regard to cell X. One might say for example, that when cell Y loses water to the sugar solution, the pressure difference between the two cells increases, resulting in even greater compression of cell X. Actually the pressure difference does increase, but what has been ignored in such an argument is the fact that decrease in volume of cell Y results in a withdrawal in space of the position at which it resists expansion of cell X. This then is the mechanism which the writer proposes to submit to explain absorption of water from a sugar solution by guard cells surrounded by living epidermal cells.

It is suggested that the experimental results indicate that the guard cells, when they are surrounded by living epidermal cells, are compressed in much the same manner as obtains in the theoretical examples, with but one essential difference. In the models the walls of the compressed cell are actually stretched, whereas in the guard cells the walls are also compressed. This latter condition was purposely avoided in the models merely for simplicity. Now it is, one should imagine, perfectly legitimate to assume that the epidermal cells lose water when a piece of epidermis is transferred from water to a sugar solution. If that is so, the position in space at which the epidermal cells resist expansion of the guard cells will change just as in the models, and, since the guard cells are compressed,



Text - figure 3. Explanation in text.

any withdrawal of intercellular pressure can only lead to one thing, viz., widening of the stomata. This would of course take place irrespective of the concentration of the external solution, but what is more important, if the osmotic pressure of the contents of the guard cell is greater than the osmotic pressure of the external solution, the protoplast of the guard cell will absorb water from that solution. Let it be stressed again that actual separation of the cells, one from the other, is not involved.

Of considerable interest is the behaviour in Zebrina. Here we have evidence that guard cells surrounded by living epidermal cells may undergo an increase in volume followed by a decrease, on being passed through gradually increasing concentrations of sucrose. This means that they continue to absorb water from a sugar solution, even when subjected to an inwardly directed pressure of their own cell wall under tension. That this should take place is quite conceivable and may be interpreted as follows. In a certain concentration of sucrose the conditions are such that the guard cell walls are neither stretched nor compressed, and the turgor pressure within the guard cells is exactly balanced by the intercellular pressure, or resistance to expansion, offered by the surrounding epidermal cells. If further increase in the concentration of the external solution causes the epidermal cells to lose more water or in other words, brings about further withdrawal of intercellular pressure, then clearly enough, the guard cells can absorb more water provided the guard cell walls are extensible, and the osmotic pressure of the contents of the guard cells is

greater than that of the external solution. Now the tension in the guard cell wall increases as water is absorbed, whereas the turgor pressure within the guard cell decreases by dilution of its contents, and also as a result of increasing concentration of the external solution, which automatically reduces the turgor pressure. Consequently, beyond a certain concentration of the external solution, the turgor pressure of the guard cells will become less than the sum of the forces (wall pressure + intercellular pressure) tending to squeeze water out, and the guard cells will then lose water.

Differences such as are found between Anthericum and Zebrina, and also within a single species, as in Zebrina, are to be expected. The balance is a delicate one and differences might well occur, even in the same species at different times and from different locations, depending upon variations in osmotic pressure of the contents of the cells, in the degree of compression of the guard cells, and the volume changes possible in the epidermal cells. However, it is suggested that the mechanism is the same in all cases, and of the nature just described. It will be recognised that a very important factor in the action of intercellular pressure is now revealed. The position in space at which intercellular pressure is applied, or at which it functions as a resistance to expansion of adjacent cells, must be taken into account, under conditions where water exchange is taking place.

DISCUSSION

Two suction pressure equations, incorporating the concept of intercellular pressure (A), have already been advocated;

(1) $S_p = O_p - (W_p + A)$, suggested by Ernest, and (2) $S_p = O_p - (W_p \pm A)$, suggested by Ursprung. The present writer has indicated another relationship, viz., $S_p = O_p - (A \pm W_p)$, which has already been discussed in terms of theoretical models. It is suggested that this is the relationship that would have to be applied to stomatal behaviour, as revealed in this paper. At equilibrium with water, when the guard cells are compressed, $S_p = O_p - (A - W_p)$, ($-W_p$) since the wall pressure is negative relative to the guard cell, and so tending to cause water to enter the guard cell. When the guard cell wall is neither stretched nor compressed, and the turgor pressure (T) within the guard cell is exactly balanced by the intercellular pressure of the surrounding cells (A), $S_p = O_p - A$. This relationship is indicated in the experiments with Zebrina, but it must also occur at some stage during normal stomatal opening, in every case where the guard cells are compressed. Finally, if the guard cell wall is stretched, so that it exerts an inwardly directed pressure on the contents of the guard cell, and if the stomata are subjected to intercellular pressure, as when they are open under normal conditions, $S_p = O_p - (A + W_p)$. The writer confesses that he has been unable to conceive of any situation that could be expressed in terms of the relationship, $S_p = O_p - (W_p - A)$, as indicated by Ursprung (6), and is of the opinion that such a rela-

tionship is untenable, simple because zero is the absolute minimum value for A. It is therefore suggested that a general equation incorporating intercellular pressure (A) should be written, $S_p = O_p - (A \pm W_p)$, ($+ W_p$) where wall pressure is positive tending to squeeze water out, and ($- W_p$) where wall pressure is negative, relative to the cell, tending to cause absorption of water.

Now certain difficulties attendant on the incorporation of intercellular pressure into the suction pressure equation have been revealed. It has been shown that one has to differentiate between intercellular pressure exerted by a cell as a whole, and that exerted by the cell contents. Moreover, the cell walls of dead adjacent cells may have the same effect as intercellular pressure, in that they may prevent full expansion of a living cell. It has also been pointed out that in terms of the amended suction pressure equation, one has to conceive of both wall pressure and intercellular pressure exerted upon the cell, as varying at different points in the same cell at the same moment, and it may be impossible to ascribe to a cell as it occurs in situ within a tissue a wall pressure (W_p) or an intercellular pressure (A), characteristic of the cell as a whole. A question of some considerable importance arises at this point. If a cell is isolated from a plant, and the intercellular pressure (A) exerted upon it before isolation estimated by determining the osmotic pressure of a sucrose solution in which the cell retains its original volume, is it legitimate to ascribe to the cell, as it occurs within the plant, the intercellular pressure determined as above, and a wall

pressure (W_p) estimated therefrom as being equal to the turgor pressure (T) of the cell less the intercellular pressure, (i.e. $W_p = T - A$). The writer doubts very much if this is legitimate, in view of what has just been said about the impossibility of ascribing a uniform wall pressure and intercellular pressure, to what is really a very common type of plant cell, viz., one having contact with other cells and also with intercellular spaces. On the other hand, it is just possible that such a procedure, whereby what may be called an average wall pressure and intercellular pressure are ascribed to a cell, might be useful and indeed accurate for the purpose of calculating other equilibria, resulting from known changes in one or more factors obtaining at some particular equilibrium. However, it will probably be admitted that the usefulness of an exact knowledge of the relationships obtaining at one equilibrium, as an aid towards an accurate deduction of the relationships at some other equilibrium, is clearly limited, even if the changes initiating formation of the new equilibrium are exactly known. It would be difficult enough in the case of a single isolated cell, and much more so in the case of a cell in situ within a plant.

In the plant cell turgor pressure (T) is definitely characteristic of the cell as a whole, and theoretically measurable. Numerically, turgor pressure is equal to the force or forces preventing further absorption of water by the cell at any equilibrium, (i.e., $T = A \pm W_p$), but it acts in the opposite direction, and it might not be considered logical to substitute T for $A \pm W_p$ in the suction pressure equation,

since turgor pressure is by definition a measure of the tendency for water to enter the cell. However, there is still another possibility. When a cell is isolated from a fully turgid tissue, the osmotic value of its contents is not thereby changed, so that if further absorption of water takes place it can mean but one thing, viz., that the cell wall has in one way or another become more extensible. Why not then conceive of intercellular pressure as modifying the extensibility of the cell wall, not the wall pressure, and let the suction pressure equation retain the simpler form of $S_p = O_p - W_p$? By modification of the extensibility of the cell wall, the writer does not imply that changes in the inherent physical or chemical characteristics of the cell wall take place. For example, death of cell X in the system depicted in text - figure 1 renders the walls AB and CD together more extensible, and complete removal of cell X renders the single wall CD still more extensible. This would avoid the difficulty of having to conceive of wall pressure and intercellular pressure as variables, in relation to a single cell at one and the same moment. It is true that the simple relationship, $S_p = O_p - W_p$, does not indicate that intercellular pressure may be involved. On the other hand, it is undoubtedly equally true that in any tissue, composed of cells with extensible cell walls, intercellular pressure is a normal factor in the force counter-balancing the tendency for water to enter the cell. Nevertheless, it is clearly important that the action of intercellular pressure be thoroughly understood. How otherwise could one understand stomatal behaviour as revealed in the foregoing experiments?

The fundamental difficulty in incorporating the concept of inter-cellular pressure into the suction pressure equation lies, in the opinion of the writer, in attempting to ascribe to a cell, when that cell is a component of a tissue, properties of its cell wall characteristic of the cell, only when it is completely isolated from the tissue. Actually, the writer does not feel greatly concerned as to whether or not intercellular pressure (A) should be incorporated into the suction pressure equation. The suction pressure relationship of itself, cannot pretend to say more than that if at any time, the force tending to cause water to enter a cell is greater than the force or forces tending to prevent absorption of water, then that cell will definitely absorb more water, and the difference between these two forces is a measure of the force with which water will begin to enter the cell. The converse relationship is also implied. More important than the desirability of indicating this relationship in the form of an equation, is the necessity for a thorough understanding of the different forces involved at any equilibrium, and the changes that occur when equilibrium is disturbed. Thereafter, expression of the relationship between the forces in the abbreviated form of an equation should be comparatively simple.

There is at least one other point that calls for discussion. The general opinion is that even when the stomata are shut, the osmotic pressure of the guard cells is not less than that of the epidermal cells. The general belief is based upon estimations of osmotic pressure at incipient plasmolysis, which, as has been already

suggested in this paper, may be very far from indicating, that at that point, wall pressure has just been reduced to zero. In this regard the error would tend to be greater in the case of guard cells than epidermal cells. In view of the results obtained in this research, it is suggested that when the guard cells are compressed, (by compression it is implied that the volume of the guard cell is less than that obtaining at incipient plasmolysis), the osmotic pressure of the contents of the guard cells must be less than that of the epidermal cells. How otherwise could the guard cells be compressed, if the posture of cell walls between adjacent protoplasts is a function of pressure differences in these protoplasts at any equilibrium?

SUMMARY

1. It is suggested that the suction pressure of a cell (S_p) should be defined, not as the force per unit area with which the cell tends to absorb water, but as the force per unit area with which the cell begins to absorb water, when it is immersed in pure free water. Turgor pressure (T), which is the actual pressure exerted upon the cell wall by the cell contents, can be defined as the force per unit area with which water tends to enter the cell at any particular moment.

2. The behaviour of stomatal guard cells of certain plants, when a piece of epidermis, taken from a leaf that has been kept in darkness for a considerable time, is passed from water through a gradually increasing concentration of sucrose, is described. Guard

cells surrounded by living epidermal cells absorb more water, on being transferred from distilled water to suitable concentrations of sucrose. The mechanism is interpreted in terms of a withdrawal of the intercellular pressure or resistance to expansion offered by the surrounding epidermal cells, as a result of loss of water by these cells. In water the intercellular pressure of the surrounding epidermal cells is great enough to compress the guard cells to a volume less than that obtaining at incipient plasmolysis. When the epidermal cells immediately adjacent to the stomata are dead, the guard cells lose water on being transferred from water to solutions of sucrose.

3. The concept of intercellular pressure is examined, and certain difficulties attendant on the incorporation of this concept into the suction pressure equation discussed. If intercellular pressure (A) is to be retained in the suction pressure equation, it is suggested that the equation take the form of,

$$Sp = Op - (A \pm Wp), \quad (+Wp) \text{ where wall pressure is positive relative to the cell, tending to squeeze water out, and } (-Wp) \text{ where wall pressure is negative relative to the cell, tending to pull water in.}$$
It is indicated that the simpler relationship, $Sp = Op - Wp$, might quite well be retained, if intercellular pressure is conceived of as modifying the extensibility of the cell wall, not the wall pressure as is implied in the amended suction pressure equation incorporating intercellular pressure.

4. The general belief that even when the stomata are shut, the osmotic pressure of the contents of the guard cells is usually slightly higher, certainly not lower, than that of the epidermal cells, is questioned. It is suggested that if the guard cells are compressed, the osmotic pressure of the contents of the guard cells must be lower than that of the epidermal cells.

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H. Seab

CAMBIAL ACTIVITY, ROOT HABIT AND
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BY
A. B. BROWN

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CAMBIAL ACTIVITY, ROOT HABIT AND SUCKER SHOOT DEVELOPMENT IN TWO SPECIES OF POPLAR

By A. B. BROWN

Department of Botany, University of Alberta, Canada

(With Plate IV and 6 figures in the text)

INTRODUCTION

IN 1930 Priestley (9), as the result of an able review of the literature in conjunction with his own observations, suggested the general rule that in trees cambial activity recommences within the buds in the spring and spreads from there basipetally down the branches. This subject and its wider relationships were further discussed by the same investigator (10) in his presidential address to Section K of the British Association for the Advancement of Science in 1932, and still more recently Priestley, Scott and Malins (11), using a new technique, claim to have confirmed this phenomenon in the case of thirty species of hardwoods and six species of softwoods.

Fewer observations are available on cambial activity in the root system of trees. Gulbe (4), working with a group of trees which included a species of poplar (*Populus tremula*), found that it started either with the swelling of the buds or after the trees had leaved out and that it first became apparent in the young twigs during the first half of May, whence it spread basipetally down the trunk, reaching the thick proximal parts of the roots during the first half of June, and then continued into the thin distal parts of the roots during the second half of June. He also found that cambial activity ended in the same order, stopping first of all in the young twigs during the second half of August and ceasing in the distal parts of the root system during the second half of October, after which the roots remained completely dormant throughout the winter. Von Mohl (6),

on the other hand, observed uninterrupted radial growth of roots during the winter but at a very much slower rate as compared with other seasons. These results were confirmed by Resa (12) and Engler (3) in the case of deciduous trees, but no activity was observed in conifers during the winter season. Recently Cockerham (2) has observed slow continuous cambial activity throughout the year in the thin distal region of the roots of *Acer pseudoplatanus* upon which is superimposed, in the more proximal or middle region, the much more vigorous basipetal influence reaching it from the trunk. The dormant condition of the cambium, such as is found in the stem, was only observed in the roots for a short distance from the base of the trunk. Cambial activity was initiated in the new elongation growth within the bud and spread basipetally down the trunk and continued in the same direction into the root, as far as it went. He also found that cambial activity ceased in the same order. Another detailed study has been made by Wight (15). He worked with specimens of *Pinus sylvestris* and observed a sudden resumption of radial growth uniformly along the trunk accompanied simultaneously by a slow basipetal spread down the branches from within the buds. He did, however, observe later, when about $\frac{1}{2}$ in. of new elongation growth had formed, that there was a falling gradient of xylem formation and lignification down the trunk. Cambial activity in the root did not become apparent until six weeks after its inception in the trunk, but again it was resumed uniformly along the length of the root. Cessation of cambial activity was found first of all at the root apices from which it proceeded backwards towards the proximal region of the roots. In the aerial parts of the tree the oldest branches ceased growth first, and the final cessation of growth took place in all parts of the branch about the same time. The uppermost branches, the whole of the trunk and the proximal parts of the root ceased growing last of all and at about the same time in all parts. These observations on *Pinus sylvestris* do not agree very well with Priestley's general statement, but Wight has admitted in his paper that, although he considered his conclusions justifiable, his data did not preclude the possibility of an exceedingly rapid basipetal "wave of initiation" of radial growth which spread down the trunk and continued in the same direction along the roots.

Various other investigators have observed elongation or extension growth of roots to continue far into the winter and in some cases throughout the winter, but there is no evidence of a causal relationship between elongation growth and radial growth in roots, such as

is known to exist between elongation growth and radial growth in stems. Wight⁽¹⁵⁾ has examined the growing apices of roots of *Pinus sylvestris* in June and September and found no evidence that growth in length of the root had any effect upon radial growth, beyond a distance of 2 in. behind the new growth. Reference should be made to a paper by Stevens⁽¹⁴⁾ for a useful summary of the literature on growth in roots.

MATERIAL

The observations recorded in this paper were made on trees growing in the vicinity of Edmonton, Alberta, Canada. In this region there are two native species of poplar, viz. *Populus tremuloides* Michx., the aspen, and *Populus balsamifera* L., the balsam poplar. The state of affairs in both these species was found to be identical; so henceforth the material will be referred to simply as poplar, but the bulk of the work was done with *Populus tremuloides* which is the more common of the two. The trees studied varied in age from one to twenty years. In this area, the usual and in all probability the only successful method of reproduction in poplar is by means of sucker shoots. Large quantities of viable seed are produced each year by the female trees, but successful seedlings rarely become established due to unfavourable environmental conditions. Suckering of poplar is particularly striking in certain areas of this province under certain conditions. The exceedingly rapid and uniform regeneration of poplar over burned areas, or in areas where the trees have been cut down in the process of clearing, is due entirely to the development of root suckers, with the exception of some suckers arising from the base of stems. When land which has been under cultivation is left undisturbed for any length of time progressive suckering from bordering poplar stands takes place very rapidly. Progressive suckering is also very strikingly apparent in the rolling park-land region, south-east of Edmonton, an area which has recently been described by Moss⁽⁷⁾. On the north-facing slopes of the knolls there occur, commonly, groves of aspen poplar surrounded by typical prairie. These groves as such, in the area in mind, originated as root suckers, after a severe fire about twenty-five years ago. The innermost part of the stand consists of trees, more or less of equal age, the original sucker shoots in fact, now from twenty to twenty-five years old. Towards the margin of the stand the trees become on the whole progressively younger, and just beyond the margin there may be a zone of prairie varying in width from 10 to 20 ft. in which are to be found diffusely

scattered poplar suckers, from one to five years old, spreading more or less radially outwards in the same centrifugal fashion with respect to age. Actually all the local poplar stands as they exist to-day have been derived from suckers, and it is for this reason that we find large numbers of trees on a common root system. The number of trees in a stand is usually greatly in excess of the number of true individuals present.

Most of the material studied was obtained from locations where poplar was spreading into unoccupied territory, for example grassland or land which had been cleared of poplar and then left undisturbed. This type of material was selected because it could be more easily excavated than poplars growing in a close stand, and also because it was found to be more suitable for the observation of certain important points.

ROOT HABIT

The root system of the aspen poplar has already been described by Baker(1) and by Kittredge and Gevorkiantz(5), but these investigators do not mention certain important features which were very apparent in the material studied by the author. It is comprised of a shallow-seated, horizontally spreading system of scaffold roots upon which are borne the thinner fibrous feeder roots. For long distances the scaffold roots may be within a few inches of the surface of the soil, and the bulk of the underground parts is to be found in the first two feet of soil. Reference to Text-fig. 1 will illustrate the important features. The drawing is not made to scale, the length being reduced to a much greater extent than the width of the roots. This diagram might well represent a system, from 20 to 30 ft. long, in which the greatest root width is $1\frac{1}{2}$ in. and the smallest $\frac{1}{16}$ in., and in which the oldest shoot is about ten years and the youngest one year old. The sucker shoots are in section, the scaffold roots in surface view, whereas the fibrous feeding roots are not depicted at all. Attention should be paid to the following points. The sucker shoots borne on the young distal portions of the root system are the most recently formed, and on the whole they become progressively older as the older parts of the roots are approached. But it is not uncommon to find a young sucker shoot growing from a portion of the root system bearing much older shoots quite close by. Moreover, some roots run for long distances, up to 15 ft. for example, without giving rise to any sucker shoots at all and without varying appreciably in width throughout that length. What is perhaps the most striking feature of all in such

a root system is the fact that distal to the sucker shoots the root upon which the sucker arose is with few exceptions markedly thickened. This thickening becomes even more obvious if the tissues external to the wood are peeled off (Pl. IV, fig. 1). The grain of the wood can then be followed, and is found to run down that side of the shoot which is



Text-fig. 1. Scaffold root system of poplar showing progressive suckering. The sucker shoots are seen cut transversely and the scaffold roots in surface view.

distal and continues smoothly along the root on the distal side in the longitudinal direction. But on the proximal side the grain undergoes a sudden change in direction. In the region common to both root and shoot it divides and turns sharply through an angle of 90° to right and to left, after which the grain swings round and again runs longitudinally along that part of the root distal to the sucker shoot. This

type of behaviour was found to be absolutely invariable under normal conditions. In a longitudinal radial section of the sucker shoot and that part of the root to which it is attached, the wood elements are all cut longitudinally or but slightly obliquely on the distal side, whereas on the proximal side, in that region common to shoot and root, they are cut completely transversely over a short distance (Text-fig. 5). Now if two suckers of approximately equal age arise close together on the same root, or if a younger sucker develops close but distal to an older sucker on the same root (Text-fig. 1), it is usually the case, so far as the more distal sucker is concerned, that that part of the root distal to it may not differ appreciably in thickness from that part of the root proximal to it, and for an obvious reason, viz. that the proximal part of the root is being thickened by the activity of the sucker shoot immediately proximal to it. This type of thing is found commonly in close even-aged stands. But examination of the grain of the wood in such cases will show that it behaves just as has been described above, i.e. it runs longitudinally down the shoot and continues longitudinally only along that part of the root distal to it. Another point of interest is that the development of sucker shoots often leads, after a time, to the production of new branch roots close to them, but invariably these new roots arise either from the base of the shoot itself or from that part of the parent root immediately distal to it.

THE INITIATION AND CESSATION OF CAMBIAL ACTIVITY

In this study xylem formation was adopted as the chief criterion of cambial activity. It was observed first of all, about the second week of May (1933), just below the expanding foliar buds, whence it spread basipetally down the shoot, and by the end of May a falling gradient of xylem formation and lignification had been established which in the case of young trees had reached the base, but in the case of some trees about twenty years old which were examined, xylem formation faded out some considerable distance above ground-level. Further study showed that this "flow" (a term to be discussed later) of cambial activity continued as such into the root system but only in a very definite direction, viz. along that part of the root distal to the shoot. An attempt has been made to depict this behaviour in Text-fig. 2, where the cross-hatching indicates newly formed xylem. It will be observed that from the base of every sucker shoot there has been established a falling gradient of xylem formation along that part of the root distal to the sucker, i.e. in the acropetal direction. It is also

true that immediately proximal to the shoot a falling gradient of xylem formation in the opposite direction was evident, but as shown in the diagram it was very feeble, of limited extent, and would soon be met by an acropetal "flow" from the first sucker shoot proximal to it. Material at the stage of development just described was found during the second half of June in the case of younger trees. No exception to this sequence of events was observed except locally, in the case of root injury, particularly grub injury, where cambial activity had been initiated at isolated points and quite independent of the acropetal "flow" from the shoots. It was found exceedingly difficult to obtain the ultimate growing points of the scaffold roots, but in many instances the roots were excavated until they were less than 1 mm. in diameter and no evidence was obtained of cambial activity, independent of the acropetal development from the base of the shoot. This was confirmed by examination of a few two-year-old seedling trees in which the growing points of the roots were obtained. Here roots were found which for several inches behind the meristematic apex showed nothing but primary wood and the system as a whole, no indication of cambial activity independent of the stimulus emanating from the shoot.

As regards cessation of cambial activity, all the evidence pointed to the conclusion that it took place in exactly the same order as its inception. Examination of material of different ages from the middle of August onwards showed quite definitely that cambial activity ceased first of all in the youngest twigs and from there basipetally down the shoot into the root system, in which activity ceased in the same acropetal fashion as it started. The criteria adopted as indicating cessation of cambial activity were the fact that the tissues external to



Text-fig. 2. Portion of scaffold root system of poplar bearing two sucker shoots, to show falling gradient of xylem formation. Cross-hatching indicates newly formed wood.

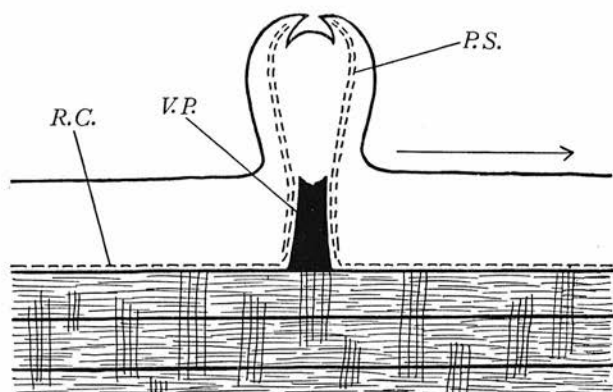
the cambium would no longer slip over the wood, and the presence of mature lignified xylem immediately adjacent to the cambium so that an even, clear-cut division between these two tissues was visible under the microscope. At the same time the appearance of the cambium itself was also studied before coming to any conclusion as to whether or not it had ceased dividing. It was difficult to decide just when cambial activity had ceased throughout the root system, but during the first half of September cambial activity could only be discovered in the thin distal portions of the roots where the diameter of the wood was not more than 0.5 mm. In view of Cockerham's(2) recent observation that cambial activity continues throughout the year in all but the more proximal parts of the roots of *Acer pseudo-platanus*, particular attention was paid to the appearance of the cambium in the roots of poplar during late autumn, but no differences were noted between the resting stem cambium and the root cambium at any point that would suggest anything but that the root cambium was dormant also.

There are certain items of interest worthy of mention at this point. As would be expected, it was found that cambial activity may have ceased in that part of the root distal to a sucker shoot, whereas in the older part of the root proximal to the shoot activity was still evident. However, observations were not so easily made of this type of thing as of the reverse, found when cambial activity was beginning, but this stage could be obtained, if pains were taken to secure at the right time material in which sucker shoots were located at a considerable distance from their nearest neighbours on the same root. It was found to be generally the case that the "flow" of cambial activity reached the roots earlier from younger than from older trees, and consequently the younger, more distal parts of the roots showed thickening before the older proximal parts of the same system bearing older suckers. In the same way the cambium had ceased to divide throughout the aerial parts of younger trees on the whole earlier than in older trees, with the result that in younger parts of the roots the cambium might be dormant, whereas in older more proximal parts of the same system it might still be active. Another point was noticed which at first proved to be rather confusing. It was not uncommon to find, in that part of the root immediately distal to a shoot, that growth was quite excentric, being more rapid on the lower side than the upper, and material was found in which growth appeared to have stopped on the upper side while it still continued on the lower side, whereas at points more distal and in the root proximal to the shoot cambial

activity had ceased both above and below. However, the explanation is probably quite simple. In the region of excentric growth cell division is exceedingly rapid and lignification lags behind, with the result that even after the cambium has ceased to divide several layers of immature, non-lignified wood elements may be found within it, thus giving rise on occasions to a localised region where the xylem merges gradually into the cambium, instead of being sharply delimited from it.

ORIGIN AND DEVELOPMENT OF THE SUCKER SHOOT

The sucker shoot is derived from a bud arising in the root phellogen, and since the phellogen, in the species worked with, proved to be pericyclic in origin, the sucker bud is really an endogenous structure. At an early stage in development there is a small nidus

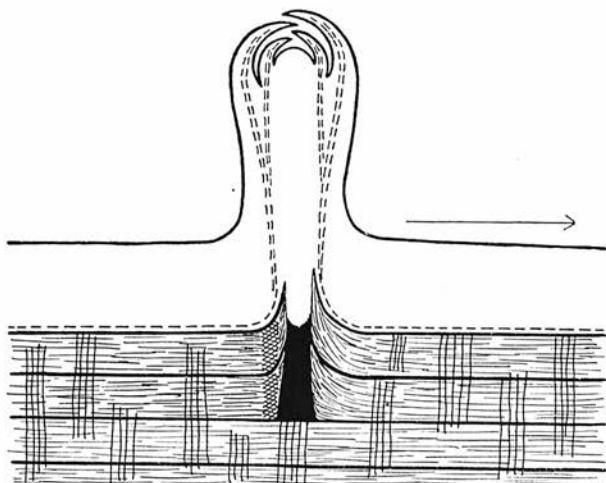


Text-fig. 3. Longitudinal radial section of sucker bud and root where vascular connection between bud and root has been made just as the root cambium became dormant. *P.S.*=procambial strand. *V.P.*=vascular peg. *R.C.*=root cambium. The arrow points in the distal direction.

of meristematic cells just within the cork and without any vascular connection whatsoever with the conducting system of the root. From this point development proceeds in two directions. Gradually a definite meristematic apex is established, giving rise in the usual way to enclosing leaf initials with their associated procambial strands, and at the same time vascular connection with the root is attained by the direct modification of a more or less cylindrical peg of bast cells, stretching from just below the bud to the central woody core of the root, to form pitted or scalariform tracheids. The ring of

procambial strands encloses this vascular peg and joins up below with the root cambium. This stage in development is depicted in Text-fig. 3, where vascular connection between bud and root has been made just as the root cambium became dormant. The bud and root are both cut in radial longitudinal section.

Text-fig. 4 illustrates the state of affairs two years later. The bud is now larger and more leaf initials have been formed, but in the interval two new rings of wood have been laid down by the root cambium. Notice first of all that this new wood encloses the vascular



Text-fig. 4. Longitudinal radial section of sucker bud and root, two years after the attainment of vascular connection between bud and root. Cross-hatching indicates xylem cut more or less transversely. The arrow points in the distal direction.

peg, and also that on the proximal side of the peg the wood elements suddenly become transverse, whereas on the distal side they are longitudinal at all points. Longitudinal sections which cut the vascular peg transversely and the root xylem tangentially (Pl. IV, figs. 2 and 3), give further information as to the orientation of the tissues at this stage of development. The picture now seen is that of a "flow pattern", essentially similar to that obtained when a liquid flows slowly round a cylindrical obstruction. Immediately proximal to the peg the wood elements turn sharply through an angle of practically 90° in the tangential plane, so that in radial longitudinal sections of the root these cells are cut transversely (Text-fig. 4), whereas on the

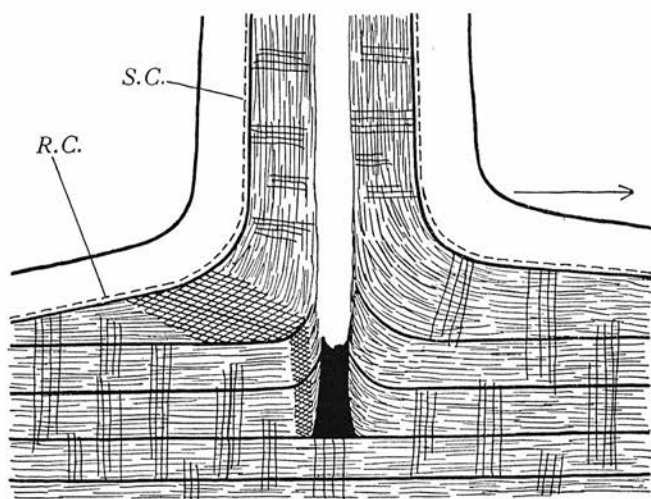
distal side the cells turn in again but slightly, so that they are cut almost completely longitudinally or at the most but slightly obliquely in longitudinal radial sections of the root. Sections through the phloem in the same plane reveal a similar state of affairs, and without a doubt this indicates the orientation to be found in the cambial layers themselves.

It should be remembered at this point that the vascular cambium of the root is continuous with the procambial strands in the sucker bud. By this time the procambial strands form a complete cylinder in which protophloem and protoxylem have begun to differentiate, so that cells common to both the root cambium and the bud cambium (ultimately the sucker shoot cambium) are involved in the reorientation of the plastic cambial layers on the proximal side of the bud.

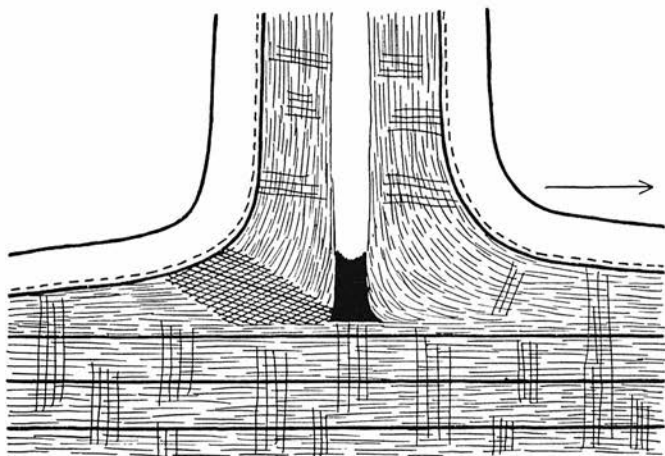
Moreover, it will be observed (Text-fig. 3) that a certain amount of xylem continuous with the root xylem has differentiated around the base of the bud. That this is really a manifestation of root cambial activity, rather than developmental activity of the bud itself, is shown clearly in cases where for some reason or other a sucker shoot has died, when it will be found that a similar wedge-shaped mass of wood is laid down around the base of the dead sucker shoot just where it joins the root.

Text-fig. 5 shows the final stage in development, in this case after another year has elapsed. During this last year the bud has gone ahead to form a shoot above ground, so that the drawing represents a radial longitudinal section of a one-year-old sucker shoot and the root upon which it arose. Notice again that the wood elements are cut transversely immediately proximal to the shoot, in that region common to both shoot and root, whereas elsewhere they are cut longitudinally. Another point that can be observed in the drawing is that the amount of wood laid down in the root distal to the sucker during the year is decidedly greater than that on the proximal side. The course of events in following years is a repetition exactly of what has already been described for the year in which the bud developed into a sucker shoot.

Sucker buds in all stages of development can be found on poplar roots at any time during the year. In the foregoing description of the development of the sucker bud a particular case has been selected, namely, where vascular connection between bud and root was completed just as the root cambium became dormant. It is important to note that this is not the only possibility, indeed it may happen but seldom. Actually, observation shows that vascular connection



Text-fig. 5. Longitudinal radial section of root and a one-year-old sucker shoot derived from the bud depicted in Text-fig. 4. *R.C.*=root cambium. *S.C.*=shoot cambium. Cross-hatching indicates xylem cut more or less transversely. The arrow points in the distal direction.



Text-fig. 6. Longitudinal radial section of root and a one-year-old sucker shoot, where vascular connection between bud and root was completed after cambial activity had recommenced in the root. This was followed by development of the bud into a shoot, during the same growing season. Cross-hatching indicates xylem cut more or less transversely. The arrow points in the distal direction.

between bud and root may be made at any time during the growing season, and that subsequent behaviour is along similar lines in all cases. The bud may not develop into a shoot for many years after vascular connection has been made with the root, but it is probably not quite true to say that the bud lies dormant, since it continues to form new leaf initials and on the whole increases in size. On the other hand, cases have been observed (Text-fig. 6) where vascular connection was completed after cambial activity had recommenced in the root, and development into a shoot followed during the same growing season. However, this latter type of behaviour does not appear to be common under normal conditions; usually, development is extended over a much longer period of time.

DISCUSSION

The foregoing observations have suggested to the author a conception of cambial activity in terms of a "flow". This idea of a cambial "flow" is based upon the following considerations: (1) the observation below the bud of "flow patterns" which presumably imply some type of flow mechanism for their formation; (2) the absence of anatomical evidence of any structural peculiarity of the cells, or of the architecture of the tissues that could account for the behaviour of the cells proximal to the sucker bud; (3) the fact that cambial activity in the region common to both root and bud is a manifestation of root cambial activity rather than developmental activity of the bud itself; and (4) the belief that the conception of a "flow" of cambial activity would seem to be a very convenient way of describing the observed behaviour in a more specific manner than, for example, "spread" of cambial activity.

Let us review in the light of this interpretation the example already described, where vascular connection between bud and root was made just as the root cambium became dormant. In the following spring, cambial activity spreads along the root in the acropetal direction from the base of growing sucker shoots. Now what does this acropetal spread of cambial activity involve? It means, firstly, that the cambial layers get progressively more plastic in this direction as the cell contents swell and are transformed from the gel to the sol state; secondly, that cell division follows in the same order; and thirdly, that differentiation and maturation of the xylem and phloem tissues take place in the same acropetal sequence. Just what happens when the vascular peg below the bud is encountered is beautifully illustrated by the "flow pattern" already described. At present no

attempt will be made to analyse the mechanism whereby the "flow pattern" is produced, beyond saying that the energy relationships within the plastic but structured cambial layers, or their still plastic derivative layers, are such that a reorientation takes place to form such a pattern. However, the attitude of the author is that reorientation of the cells to form the "flow pattern" is definitely a function of root cambial activity. Not only is it a function, but in some measure, at least, an expression of mechanism, interpreted in this paper in terms of a "flow" analogy. Observations of great importance in this connection are that vascular connection between bud and root may be made at any time during the growing season, and that subsequent behaviour is the same in all cases, resulting in the formation of a "flow pattern". On consideration, it is clear that these facts indicate that not only is cambial activity initiated as a "flow", but that it is maintained as such during its period of activity.

One possible objection to this conception of cambial "flow" will now be dealt with. Cambial activity in the shoots of plants is initiated within the developing buds, and it is quite probable that the sucker bud, despite its slow and prolonged development in many cases, would promote a certain amount of cambial activity below it, in its own axis, and perhaps even in the root axis. Now it may be that cambial activity emanating from the bud is governed by polarity, in this respect, that it can only develop in the morphologically distal direction in the root, and it is perhaps not inconceivable that polarity of this type would, in itself, be instrumental in the production of the "flow pattern" effect. However, any cambial activity on the part of the bud, in the region common to both bud and root where reorientation takes place, is completely masked by root cambial activity under normal conditions, particularly so if the root cambium is active when vascular connection is attained. It is extremely doubtful whether the bud exerts any influence at all in that region. Moreover, it is difficult to conceive of polarity in itself bringing about a reorientation of the cells to form the clearly defined "flow pattern" unless it were complete and unconditional, and of this there is no evidence. This point will be referred to again later in the discussion.

It is suggested that cambial activity may be expressed in terms of a "flow", but it still remains to explain the forces involved. At present no completely acceptable explanation, based on observational evidence, is forthcoming. Liquids flow from a point at which the pressure is higher to a point where the pressure is lower, and the pressure varies with the head of liquid. Obviously nothing completely

analogous to this takes place in the growing tree. There is definitely no actual movement of the plastic cambial initials from one point to another over any considerable distance. Nor is there any evidence of a basipetal or acropetal flow, in stems and roots respectively, of water from one cell to another, either in the cambium itself or in the differentiating layers cut off by it. Indeed, all the evidence points to the fact that movement of water is in the opposite direction, i.e. towards the apical growing points of the shoots. This means, of course, that the "flow pattern" is orientated in the opposite direction to the general movement of water in the plant. The author has given much thought to a consideration of the energy relationships of cambial "flow", but hesitates to theorise at present for lack of direct observational and experimental evidence. One possible explanation will, however, be indicated. The vessel is derived from a more or less linear series of cells which, according to Priestley (10), vacuolate progressively in the basipetal direction in the shoot and acropetally in the root. Vacuolation of the vessel segment involves entrance of water into the cell, accompanied by stretching of the cell wall, or in other words by an increase in turgor pressure, and the necessary water is derived from the previously formed layers of wood within, where the general movement of water is upwards. In effect, there is what may be termed a progressive "flow" of turgor or hydrostatic pressure within the developing vessel, in the opposite direction to the general movement of water. It is clear, of course, that this "flow" of turgor pressure would only be temporary in the formation of any one vessel, but the whole process is repeated in the development of every vessel, with the result that the "flow" of turgor pressure would be maintained over a long period of time in the same relative position, i.e. just within the actively dividing cambium. Further discussion along these lines is reserved, and it is simply suggested that here we may have a "flow" mechanism capable in one way or another of causing the formation of a "flow pattern" around the vascular peg below the sucker bud.

The conception of a "flow" of cambial activity could very readily be extended to explain in an extremely simple manner the relationship between a sucker shoot and the parent root. Briefly, the hypothesis is that reorientation of the cells, during development of the bud, in the region common to both root and bud on the proximal side, may predetermine the direction of "flow" of cambial activity emanating from the sucker shoot derived from that bud, or in other words, cambial "flow" from the shoot may be actually guided along

the root in the distal direction. This interpretation is along purely physical lines and does not invoke polarity of the type already mentioned when discussing the formation of the "flow pattern". But it does not of necessity preclude polarity, which although completely masked during the bud stage, might quite well manifest itself clearly after the bud has developed into an actively growing shoot. It is significant to note, however, that configuration of the tissues just proximal to the sucker shoot is anticipated in the bud stage. Moreover, polarity, assuming it to exist, is apparently not complete and unconditional, as witness the falling gradient of xylem formation on the *proximal* side of sucker shoots during the earlier part of the growing season. It would be interesting, in this connection, to isolate pieces of root system bearing only young buds which had not yet attained vascular connection with the parent root, and from which all shoots had been removed. However, such an experiment, simple as it is in theory, would be very difficult to carry out with poplar, as far as the author's knowledge of that material goes. But it is hoped that future work, of an experimental nature, will help towards a more complete understanding of some of the problems indicated in this paper.

Some recent experiments by Snow⁽¹³⁾ have been interpreted as indicating that cambial activity is dependent upon the production of a hormone by the leaves. The present work neither confirms nor refutes this view, but it may be stated that in general, cambial behaviour in poplar is susceptible of interpretation in terms of production of such a hormone.

SUMMARY

1. Cambial activity, in the two species of poplar studied, is initiated within the buds, and spreads thence basipetally down the stem. From the base of the stem it continues acropetally along the roots. Cessation of cambial activity proceeds precisely in the same order as its inception.

2. Cambial activity begins during the first half of May, and the first indications of cessation can be observed during the second half of August. During the winter months the cambium remains dormant.

3. Root habit, and the origin and development of the sucker bud into a sucker shoot are described.

4. It is suggested, on the basis of a certain amount of evidence, that cambial activity may, in a general way, be interpreted in terms of a "flow".



Fig. 1



Fig. 2



Fig. 3

BROWN—CAMBIAL ACTIVITY IN TWO SPECIES OF POPLAR

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EXPLANATION OF PLATE IV

- Fig. 1. Four-year-old sucker shoot on eight-year-old root, showing grain of wood and distal thickening of root. Natural size.
- Fig. 2. Section, longitudinal tangential of root and transverse of vascular peg below the sucker bud to show flow pattern effect. $\times 15$.
- Fig. 3. As Fig. 2. A different preparation more highly magnified. $\times 45$.
- In Figs. 2 and 3 the tissues above and below the vascular peg are respectively proximal and distal.

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